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Metabolomic Profiling in Inflammatory Bowel Disease

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List of Abbreviations

5-ASA	5-Aminosalicylic Acid
6-MP	6-Mercaptopurine
α1-AT	Alpha 1-Antitrypsin
ACCA	Anti-Chitobioside Carbohydrate Antibodies
ADP	Adenosine Diphosphate
AI	Activity Index
AIEC	Adherent Invasive Escherichia Coli
ALCA	Anti-Laminaribioside Carbohydrate Antibodies
AMCA	Anti-Mannobioside Carbohydrate Antibodies
AMG	Alpha 2-Macroglobulin
ANCA	Anti-Neutrophil Cytoplasmic Antibodies
Anti-C	Anti-Chitin Carbohydrate Antibodies
Anti-CBir 1	Bacterial Flagellin Antibodies
Anti-I2	Bacterial Sequence I2 Antibodies
Anti-L	Anti-Laminarin Carbohydrate Antibodies
Anti-OmpC	Anti-Outer Membrane of Porin C
API	Atmospheric Pressure Ionisation
APC	Antigen-presenting Cell
ASCA	Anti-Saccharomyces Cerevisiae Antibodies
ATP	Adenosine Triphosphate
ATG16L1	Autophagy-related 16 Like 1 Gene
BAE	Balloon Assisted Enteroscopy
BCFA	Branched Chain Fatty Acid
BMI	Body Mass Index
CAI	Clinical Activity Index
C-ANCA	Cytoplasmic Anti-Neutrophil Cytoplasmic Antibodies
CARD15	Caspase Recruitment Domain-containing Protein 15
CaSR	Calcium Sensing Receptor
CCK	Cholecystokinin
CD	Crohn’s Disease
CDAI	Crohn’s Disease Activity Index
CDEIS	Crohn’s Disease Endoscopic Index of Severity
CE	Capsule Endoscopy
CID	Collisional Induced Dissociation
CO	Carbon Monoxide
CpGs	Cytosine-Guanine Dinucleotides

CRC	Colorectal Cancer
CRP	C-Reactive Protein
DAI	Disease Activity Index
DI	Direct Injection
DNA	Deoxyribonucleic Acid
DNAm	Deoxyribonucleic Acid methylation
DSS	Dextran Sulphate Sodium
DZ	Dizygotic
EC	Endothelial Cells
ECAM	Endothelial Cell Adhesion Molecule
ECP	Eosinophil Cationic Protein
EEC	Enteroendocrine Cell
EI	Endoscopic Index
ELISA	Enzyme-linked immunosorbent assay
eNOS	Endothelial Nitric Oxide Synthase
EPX	Eosinophil Protein X
ESI	Electrospray Ionisation
ESR	Erythrocyte Sedimentation Rate
FC	Faecal Calprotectin
Fc	Fragment, crystallisable
FDR	False Discovery Rate
FL	Faecal Lactoferrin
FT	Fourier Transform
GALT	Gut-associated Lymphoid Tissue
gASCA	Anti-Saccharomyces Cerevisiae IgG Antibody
GC	Gas Chromatography
GI	Gastrointestinal
GLP	Glucagon-like Peptide
GWAS	Genome-wide Association Study
HBI	Harvey-Bradshaw Index
HC	Healthy Control
HD	Human Defensin
HDL	High Density Lipoprotein
HNL	Human Neutrophil Lipocalin
HLA	Human Leukocyte Antigen
IBD	Inflammatory Bowel Disease
IBDQ	Inflammatory Bowel Disease Questionnaire
IBDU	Inflammatory Bowel Disease Unclassified
IC	Indeterminate Colitis

ICAM	Intercellular Adhesion Molecule
ICR	Ion Cyclotron Resonance
IEF	Isoelectric Focusing
IFF	Indirect Immunofluorescence
Ig	Immunoglobulin
IKK	Inhibitor of Kappa B Kinase
IL	Interleukin
INF- γ	Interferon Gamma
iNOS	Inducible Nitric Oxide Synthase
IPAA	Ileal Pouch Anal Anastomosis
IRAK	Interleukin-1 Receptor-associated Kinase
IRGM	Immunity-related GTPase Family M Gene
IRP2	Ion Regulating Protein 2
KRAS	Kirsten Rat Sarcoma Viral Oncogene Homolog
LC	Liquid Chromatography
LDL	Low Density Lipoprotein
LPS	Lipopolysaccaride
LRR	Leucine-rich Repeat
MAdCAM	Mucosal Addressin Cell Adhesion Molecule
MALDI	Matrix-assisted Laser Desorption / Ionisation
MAMP	Microbe-associated Molecular Pattern
MAP	Mycobacterium Avium Subspecies Paratuberculosis
MAPK	Mitogen-activated Protein Kinase
MDP	Muramyl Dipeptide
MHC	Major Histocompatibility Complex
MMP	Matrix Metaloproteinase
MPO	Myeloperoxidase
MS	Mass Spectroscopy
MSI	Metabolomics Standards Initiative
MSMD	Mendelian Susceptibility to Mycobacterial Disease
MyD88	Myeloid Differentiation Primary Response Gene 88
MZ	Monozygotic
nAChRs	Nicotinic Acetylcholine Receptors
ncRNA	Non-Coding Ribonucleic Acid
NFκB	Nuclear Factor Kappa B
NHS	National Health Service
NIST	National Institute of Standards and Technology
NMR	Nuclear Magnetic Resonance
NO	Nitric Oxide

NOD2	Nucleotide-binding Oligomerisation Domain-containing Protein 2
NOS	Nitric Oxide Synthase
NSAID	Non Steroidal Anti Inflammatory Drugs
OCP	Oral Contraceptive Pill
OMGE	Organisation Mondiale de Gastroenterologie
OONO	Peroxynitrite
PAB	Pancreatic Autoantibodies
PAF	Platelet Activating Factor
P-ANCA	Perinuclear Antineutrophil Cytoplasmic Antibodies
PCA	Principle Component Analysis
PCDAI	Perianal Crohn's Disease Activity Index
PE	Push Endoscopy
PEG	Polyethylene Glycol
PGA	Physician Global Assessment
PGE_2	Prostaglandin E2
PHA	Phytohemagglutinin
PID	Primary Immunodeficiency
piRNA	Piwi-Interacting Ribonucleic Acid
PLS-DA	Partial Least Squares Discriminant Analysis
PMN-E	Polymorphonuclear Neutrophil Elastase
PPARγ	Perioisome Proliferator-activated Receptor gamma
PR-3	Proteinase 3
PRR	Pattern Recognition Receptor
PUFA	Polyunsaturated Fatty Acid
RCT	Randomised Controlled Trial
RF	Radio Frequency
RNA	Ribonucleic Acid
RR	Relative Risk
RSD	Relative Standard Deviation
SAA	Serum Amyloid A
SCCAI	Simple Clinical Colitis Activity Index
SCFA	Short Chain Fatty Acids
SE	Spiral Enteroscopy
SELDI	Surface-enhanced Desorption / Ionisation
SES-CD	Simplified Endoscopic Activity Score for Crohn's Disease
SMRS	Standard Metabolic Reporting Structure
SNP	Single Nucleotide Polymorphism
SRB	Sulphate Reducing Bacteria
STAT	Signal Transducer and Activation of Transcription

sTNFRII	Solid Tumour Necrosis Factor II
TGFβ	Transforming Growth Factor Beta
TGN	Thioguanine Nucleotide
TLR	Toll-Like Receptor
TMS	Trimethylsilyl
TNF-α	Tumour Necrosis Factor Alpha
ToF	Time of Flight
TPMT	Thiopurine S-methyltransferase
TSS	Transcription Start Site
UC	Ulcerative Colitis
UCCS	Ulcerative Colitis Clinical Score
UCDAI	Ulcerative Colitis Disease Activity Index
UHPLC	Ultra High Performance Liquid Chromatography
UK	United Kingdom
USA	United States of America
VCAM	Vascular Cell Adhesion Molecule
VEGF	Vascular Epithelial Growth Factor
VLDL	Very Low Density Lipoprotein

Declaration of Originality

I declare that I have composed this thesis, and that the research it describes is entirely my own work, unless otherwise stated.
This work has not been submitted for any other professional degree or qualification.

Diane Hildebrand
16th March 2016

Dedication

I dedicate this thesis to my family.

To Bob; for his longstanding, unwavering belief in me, and for his constant calming influence I am eternally grateful.

To my parents and brother; I would not be where I am today without you. The support and strength you give me every day is beyond compare.

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Abstract

Introduction

Inflammatory bowel disease (IBD) is a chronic gastrointestinal disorder that encompasses two major subtypes; Crohn's Disease (CD) and Ulcerative Colitis (UC). Our knowledge regarding disease pathogesis is rapidly increasing. However, these disease entities provide challenges in diagnosis, monitoring of disease activity and assessing individual response to treatment, because there is a lack of validated clinical biomarkers.

Metabolomics involves the study of numerous analytes that have very diverse physical and chemical properties and occur in a wide concentration range. Early evidence suggests there is potential for metabolomic profiling to be used in the differentiation of CD and UC. However, knowledge is limited regarding the metabolic changes seen in relation to disease activity or to medical or surgical treatments.

Aims

A metabolomics approach was taken to determine whether metabolomic profiles could distinguish between patients with CD or UC and healthy controls. We also aimed to define the relationship between metabolomic profile and disease activity, and to determine the effect of medical (anti-TNFα agents) and surgical treatment on the metabolome.

Methods

A metabolomics approach was undertaken. Serum and urine sample sets were collected from a total of 41 patients with ulcerative colitis, 43 patients with Crohn's disease, and 62 healthy controls (HC). In order to allow a comparison of metablomic profile and disease activity, 4 sample sets were taken from the same patient at 3 monthly intervals over the period of one year. Those patients undergoing either surgical or biological treatment had sample sets taken pre and post intervention. Metabolomic analysis using gas chromatography time of flight mass spectrometry (GC-ToF-MS) and ultra-high performance liquid chromatography Fourier Transform mass spectrometry (UHPLC-FTMS) was carried out on both serum and urine.

Results

Serum and urine GC-ToF-MS and UHPLC-FTMS metabolomic analyses show differentiation between UC, CD and healthy controls, most significantly in urine analyses. No significant differentiation was seen in pre- and post-surgical patients, or pre- and post-biological therapy patients. It was possible to differentiate surgical patients from healthy controls, especially in the urine analyses. Metabolite identification revealed consistently more dietary variation in the healthy controls than in the IBD patients. Significant differences ($p<0.05$) were seen between healthy controls and IBD

patients in classes of metabolites relating to the citric acid cycle and the uronic acid pathway, as well as amino acids, fatty acids and cholesterols.

The behaviour or location of disease, or the disease activity score did not appear to influence the metabolome in either serum or urine analyses using GC-ToF-MS and UHPLC-FTMS.

Conclusion

Metabolomic profiling of urine and serum in IBD may provide a novel methodology aiding both clinical diagnosis through biomarker development, and advancing knowledge of disease pathogenesis.

Lay Summary

Inflammatory bowel disease (IBD) is a chronic inflammatory condition affecting the bowel. There are two main subtypes, Crohn's disease (CD), and ulcerative colitis (UC). CD can affect any part of the digestive tract, from the mouth to the anus. UC affects the large bowel only. The reason that people get these diseases remains unclear, but the trigger is likely to be combination of factors. These are related to the genes a person has, the way in which their immune system fights infections, and the bacteria they carry in their bowel. These diseases are chronic, but tend to have periods of active inflammation in the bowel, and periods where the inflammation settles. IBD treatment essentially involves medication to reduce the inflammation in the bowel through suppression of the immune system. Surgical treatment is required if the bowel inflammation is very severe, is not responding to medical treatment, or is complicated by a perforation or impending perforation of the bowel.

CD and UC can be challenging to diagnose and differentiate. This usually requires an examination of the bowel using a flexible telescope, where small tissue samples are taken and examined carefully under a microscope. Even then the diagnosis can be unclear. This is an invasive test that is not without risk. Currently there are no less invasive tests that allow accurate diagnosis.

The monitoring of response to treatment is also a difficult field, and the only proven method is again, a telescope examination of the bowel. It is simply not practical to subject patients to this recurrently and therefore the search for a simple test, or biomarker, such as a blood or urine test that could be used to aid the diagnosis or monitoring of a patients' response to treatment, or the activity of their disease is necessary.

Metabolomics is the measurement of all of the metabolites in a substance. Metabolites are the breakdown products produced during daily bodily activities. These are measured using mass spectroscopy. By measuring these we gain knowledge of biological reactions taking place within the body. These findings can be compared to healthy people, or patients can be compared to themselves in order to better understand the biological process occurring in different phases of disease.

The aim of this study was to determine whether it is possible to differentiate between CD and UC patients, and healthy controls using metabolites measured in blood and urine samples. We also aimed to determine whether different medical and surgical treatments changed the metabolites we identified in IBD patients.

We were able to differentiate between CD, UC and healthy controls by measuring metabolites in blood and urine. Urine appears to be better at differentiating than serum. We did not find metabolites that differentiated between the activity of the disease, or where in the bowel it was affecting. It was possible to differentiate between IBD patients requiring surgery and healthy controls.

This metabolomics study has allowed us to gain insights into the biological pathways affected by IBD, and also differentiate between the subtypes. It may be possible to develop biomarkers in the future to aid diagnosis based on this technology.

1

Introduction

Introduction

Inflammatory bowel disease (IBD) is a chronic gastrointestinal disorder that encompasses two major subtypes; Crohn's Disease (CD) and Ulcerative Colitis (UC). In the UK, UC affects 146 000 people, the peak incidence of onset being between the ages of 15 and 25 years, with a second smaller peak between 55 and 65 years of age (National Clinical Guideline Centre (UK) 2013). Crohn's Disease affects 115 000 people in the UK, with up to a third being diagnosed before the age of 21 years (National Clinical Guideline Centre (UK) 2012).

Inflammatory bowel disease has been recognised since the 18th century, when in 1761 Morgagni described intestinal inflammation that we would now classify as Crohn's Disease (Kirsner 2001). Despite multiple publications by Wilks (1859), Fenwick (1889) and Dalziel (1913) regarding inflammation of the gut in the meantime, it wasn't until 1932 that Crohn, Ginzberg and Oppenheimer published the landmark paper describing terminal ileitis (Crohn, Ginzberg et al. 1932). In 1960, Lockart-Mummary and Morson described granulomatous colitis and it was understood that the condition now known as Crohn's Disease could affect the large as well as the small intestine.

Wilks is credited with the first pathological description of ulcerative colitis in a case report published in1859 (Wilks 1859). He and Moxon went on to further distinguish UC from infectious colitis in 1875 (Wilks, Moxon 1875).

1.1 Epidemiology of IBD

In North America, incidence rates for IBD range from 2.3 (Stowe, Redmond et al. 1990) to 15.6 (Blanchard, Bernstein et al. 2001) cases per 100 000 person-years for UC and from 3.6 (Kurata, Kantor-Fish et al. 1992) to 15.6 (Blanchard, Bernstein et al. 2001) per 100 000 person-years for CD. The prevalence is thought to range from 37.5 (Pinchbeck, Kirdeikis et al. 1988) to 246 (Loftus, Loftus et al. 2003) cases per 100 000 persons for UC and from 26 (Kurata, Kantor-Fish et al. 1992) to 198.5 (Bernstein, Blanchard et al. 1999) cases per 100 000 persons for CD. It is caluclated that between 7000 and 46 000 persons are newly diagnosed with UC per year, and 10 000 to 47 000 with CD per year (Loftus Jr 2004).

Sonnenberg et al (Sonnenberg, McCarty et al. 1991) studied the geographical distribution of IBD in the USA by analysing 17.5 million hospital discharges of all US Medicare beneficiaries over a 2 year period. They found that both UC and CD were more frequent in the northern states than the southern states, and in urban areas as compared to rural parts. Pinchbeck et al (Pinchbeck, Kirdeikis et al. 1988) also showed that urban areas had a higher prevalence of IBD than rural areas.

In Europe, the incidence rate of UC ranges from 1.5 (Vucelic, Korac et al. 1991b) to 20.3 (Roin, Roin 1989) cases per 100 000 person-years and from 0.7 (Vucelic, Korac et al. 1991a) to 9.8 (Kyle 1992) cases per 100 000 person-years for CD. The prevalence of UC in Europe has been reported as ranging from 21.4 (Vucelic, Korac et al. 1991b) to 243 (Rubin, Hungin et al. 2000) cases per 100 000 persons, and as 8.3 (Vucelic, Korac et al. 1991a) to 214 (Montgomery, Morris et al. 1998) cases per 100 000 persons in CD.

A recent European multicentre population based inception cohort of IBD patients has shown the median crude incidence rate per 100 000 in 2010 for CD to be 6.5 (range 0 – 10.7) in Western Europe and and 3.1 (range 0.4 – 11.5) in Eastern Europe, and for UC to be 10.8 (range 2.9 – 31.5) in Western Europe and 4.1 (range 2.4 – 10.3) in Eastern Europe (Burisch, Pedersen et al. 2014). The European collaborative study on inflammatory bowel disease reported on the apparent North-South divide in the distribution of IBD cases. They found that the highest rates of IBD come from the Scandinavian countries and the lowest from Mediterranean countries, that rates of UC in Northern centres were 40% higher than those in the South, and in CD rates in the North were 80% higher than those in the South (Shivananda, Lennard-Jones et al. 1996).

Currently it is estimated that the prevalence of IBD in the UK (United Kingdom) is approximately 400 per 100 000 people (Stone, Mayberry et al. 2003). Within Scotland itself the incidence of paediatric IBD is rising, with a statistically significant increase of 76% since the mid-1990s. The incidence of CD was 4.75/100 000/year, UC 2.06/100 000/year and IBD unclassified 1.01/100 000/year (Henderson, Hansen et al. 2012).

1.2 Demographics of IBD

In CD there appears to be a slight female preponderance, especially in the late adolescent and early adulthood periods, potentially pointing towards a hormonal influence on disease expression (Loftus Jr 2004). In UC, it seems that males are more affected (Loftus Jr 2004), and some studies actually suggest that although overall incidence is static, that male prevalence is increasing and female prevalence decreasing (Loftus, Silverstein et al. 2000).

Both CD and UC are most commonly diagnosed in late adolescence and early adulthood, although diagnosis can occur at any age. The mean age at diagnosis of CD in North America is between 33.4 and 45 years of age (Loftus, Schoenfeld et al. 2002). In UC, the mean age at diagnosis is 5 to 10 years later than in CD (Loftus, Silverstein et al. 2000, Bjornsson, Johannsson 2000). The classic bimodal distribution of diagnosis of IBD has recently been confirmed in a large population study of hospital admissions in England and Scotland with IBD. In both CD and UC the peaks were between 25 – 29 years of age, and between 75 – 79 years of age. A more prominent first peak occurred in younger patients with CD, and a more prominent second peak in older patients with UC. During the two decades studied the rate of hospitalisation for both diseases increased in all age groups, although the increase was relatively more pronounced in the elderly and especially in UC (Sonnenberg 2010).

1.3 Racial and Ethnic Implications on IBD

Traditionally, IBD is thought of as a Western disease mainly impacting on Northern Europe and North America. However, more recently the prevalence and incidence of IBD, and especially UC, is increasing throughout central Europe, Asia, Africa and Latin America. In most of these regions CD still remains a rarity (Loftus Jr 2004).

It is now suggested that the incidence of IBD amongst African Americans is similar to that of Caucasian Americans, with the prevalence of CD in African Americans being two thirds that of

Caucasian Americans. Paediatric IBD series from both North America (Ogunbi, Ransom et al. 1998) and the UK (Sawczenko, Sandhu et al. 2001) suggest that African Americans and Afro-Carribean Brits respectively have similar risks of IBD as their Caucasian counterparts. This was also shown to be the case in adults in the UK (Fellows, Freeman et al. 1990).

The study of migrant populations has been of interest in determining the impact of lifestyle and environmental factors rather than genetic factors. South Asian immigrants to the UK, and their offspring, are at increased risk of UC relative to indigenous Caucasian peoples (Probert, Jayanthi et al. 1992, Probert, Jayanthi et al. 1993, Carr, Mayberry 1999, Montgomery, Morris et al. 1999). Those who immigrated to Singapore are also at increased risk relative to the ethnic Chinese population (Lee, Fock et al. 2000). There is a higher prevalence of IBD in Jews from Europe and American when compared to those from Asia and Africa, although these differences may be decreasing (Fireman, Grossman et al. 1989, Odes, Locker et al. 1994).

1.4 Cost Implications of Care

During the last two decades hospital statistics from England and Scotland have shown a significant increase in the hospitalisation rates caused by CD and UC (Sonnenberg 2010). This has major economic implications.

It has been proposed that the total economic impact of CD was up to $US15.5 billion in the US and €16.7 billion in Europe in 2006 (Yu, Cabanilla et al. 2008). There are no equivalent studies for UC, but based on previous work the cost is likely to be similar (Bodger 2011).

The cost of IBD to the NHS (National Health Service) is thought to be £720 million per annum. This, again, is based on figures from 2006 and estimates an average of £3000 per patient per year (Luces, Bodger 2006, Cummings, Keshav et al. 2008).This has the potential to increase due to new biological therapies becoming available and bringing with them a price tag of over £1500 per infusion (based on infliximab 5mg/kg for a 75kg adult) (Joint Formulary Committee. British National Formulary (online ed.) 2011). These therapies, however, may end up as cost effective by reducing the need for inpatient care including surgery (Rutgeerts, Feagan et al. 2004, Sands, Anderson et al. 2004, Lichtenstein, Yan et al. 2005, Colombel, Sandborn et al. 2007, Feagan, Panaccione et al. 2008, Jewell, Satsangi et al. 2005).

The total economic burden of the disease is difficult to measure but given that the peak onset of disease is in the 3rd decade of life, we must assume that there is significant potential for employment prospects to be affected and earnings lost due to ill health.

1.5 Gut Anatomy and Histology

Ulcerative colitis is confined to the colon and rectum, usually progressing from the rectum proximally. Crohn's Disease may affect any part of the gastrointestinal (GI) tract from mouth to anus.

The GI tract arises initially in week 3 of embryological development, during the process of gastrulation from the endoderm of the trilaminar embryo, and extends from the buccopharyngeal membrane to the cloacal membrane. During week 4 of development, 3 distinct regions develop

extending throughout the length of the embryo: the fore-, mid- and hind-gut. These 3 divisions are later defined by their vascular supply. Endodermal cells generate the lining of the digestive tube and its glands; mesodermal mesenchyme cells will surround this tube to provide the muscles for peristalsis. The endodermal epithelium responds differently to different regionally specific mesodermal mesenchymes, forming the oesophagus, stomach, small intestine, and colon (Gilbert 2000).

1.5.1 Mucosa

The mucosa is the innermost layer of the gut. It surrounds the lumen and comes into direct contact with the chyme. It comprises 3 layers:

- Epithelium: enterocytes, goblet cells, paneth cells (small intestine only), entero-endocrine cells, M cells.
- Lamina propria: loose connective tissue, Peyer's patches, lacteals
- Muscularis mucosae: 2 layers of smooth muscle forming the boundary between mucosa and submucosa

Submucosa: connective tissue, blood vessels, submucosal (Meissner's) plexus, Brunner's glands in the duodenum only

Muscularis externa: 2 smooth muscle layers with myenteric (Auerbach's) plexus between

Adventitia: loose connective tissue

The mucosae are highly specialised in each organ of the gastrointestinal tract to deal with the different conditions throughout. The most variation is seen in the epithelium.

In the oesophagus, there is stratified, squamous, and non-keratinising epithelium, for protective purposes. There is an abrupt transition at the gastro-oesphageal junction from squamous to columnar epithelium.

In the stomach the epithelium is simple columnar. It is organised into gastric pits and glands to deal with secretions.

The epithelium of the small intestine is optimised for absorption. It is organised into plicae circulares and villi, and the enterocytes have microvilli, creating a brush border, greatly increasing the surface area for absorptive purposes. The epithelium is simple columnar with microvilli. In the ileum there are Peyer's patches in the lamina propria.

The colon has simple columnar epithelium without villi but with goblet cells.

The mucosa of the appendix resembles that of the colon but is heavily infiltrated with lymphocytes.

At the ano-rectal junction, the pectinate line, there is a sharp transition from simple columnar to stratified squamous non-keratinising epithelium for protective purposes.

Figure 1.1: Cross-section histological diagram of the GI tract (OpenStax 04/06/2013)

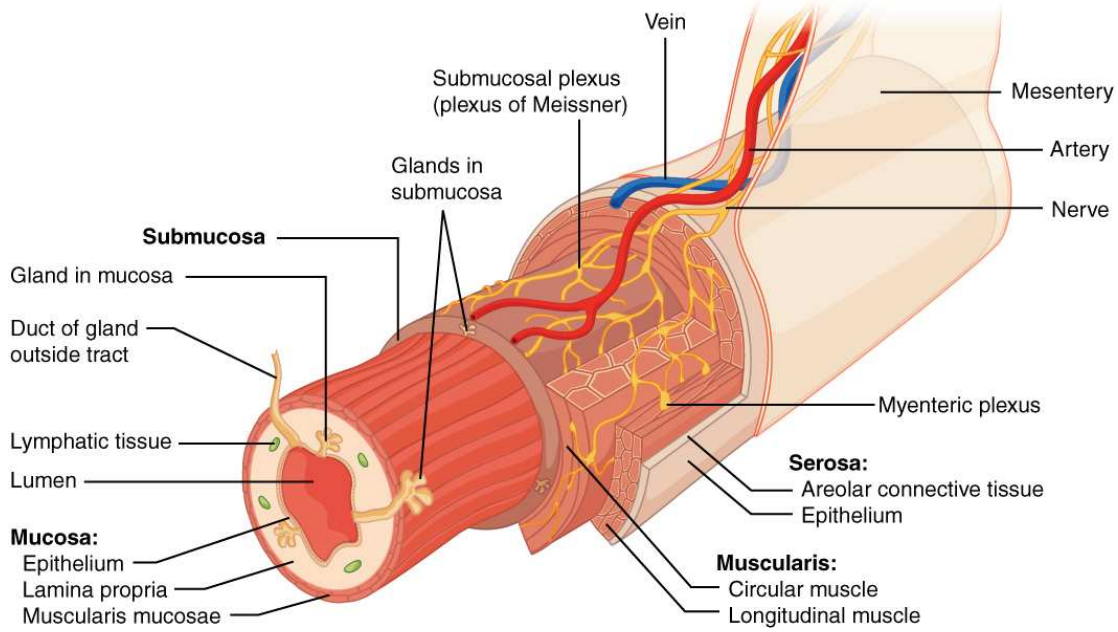
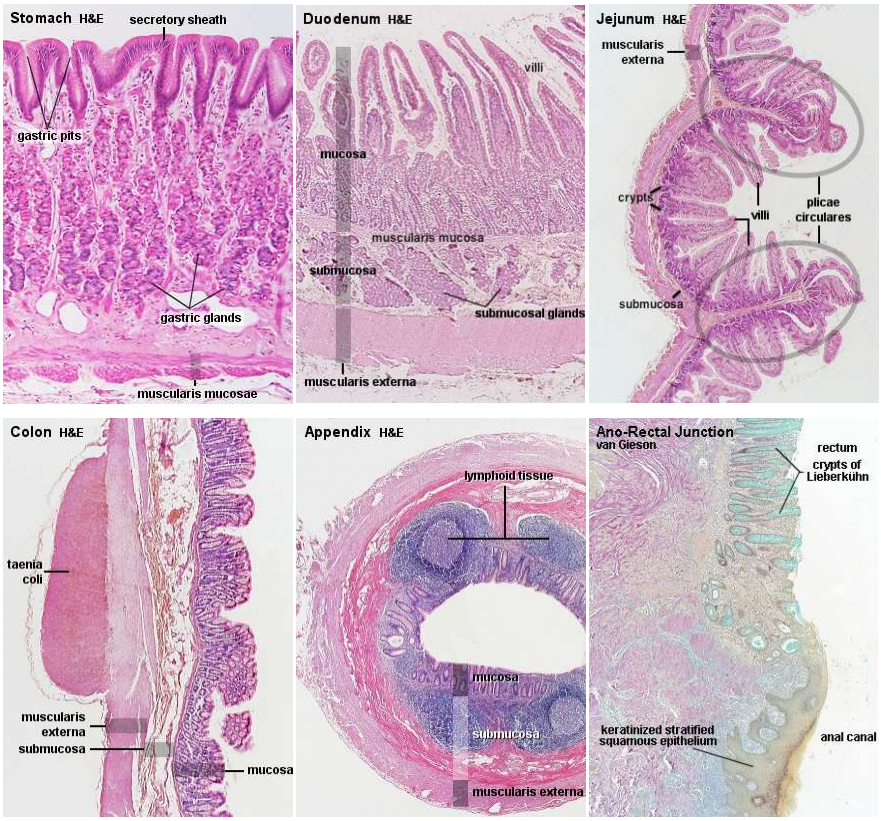


Figure 1.2: GI tract histological slides (Blue Histology - Gastrointestinal Tract 2009)



1.6 Morphology of IBD

1.6.1 Ulcerative Colitis

UC is a chronic disease of the colon, spreading proximally from the rectum. It does not affect the small bowel, other than "backwash ileitis". Endoscopically it is characterised by erythema, mucosa friability, loss of vascular pattern, and / or ulceration. The inflammatory process is limited to the mucosa and submucosa. Histologically there must be evidence of chronicity such as plasmacytosis in the lamina propria, eosinophilia, and distortion of the normal structures such as crypts and the surface mucosa. Regeneration and mucosal remodeling will result in a variety of altered shapes and sizes of crypts, and changes to the surface contours that vary from undulating to villiform. Acute active inflammation may accompany the chronic changes. Cryptitis, the invasion of crypt epithelium by neutrophils, can result in ulcers and crypt abscesses (Appleman 2008).

1.6.2 Crohn's Disease

CD, whilst also a chronic relapsing and remitting disease, is characterised by skip lesions comprising focal, patchy erosions or ulcers, vertical fissures and fistulae, which can affect the whole gastrointestinal tract. Transmural inflammation with multiple lymphoid aggregates, granulomas, plasmocytosis and lymphocytosis of the lamina propria, chronic architectural distorsion, neurtophilic inflammation including neurtophilic crytpitis and crypt abscesses are common histoligical findings, as is submucosal fibrosis and neuromuscular hyperplasia of the submucosa.

1.6.3 Indeterminate Colitis

At the time of diagnosis of IBD, 10 – 15% of patients will be diagnosed with indeterminate colitis (IC) or IBD-unclassified (IBDU). Currently we lack a diagnositic biomarker to specifiy IBD class and whilst with time > 50% of these patients will be given a formal diagnosis of CD or UC, with the majority being UC (Burakoff 2004), treatments are potentially delayed or suboptimal due to diagnostic uncertainty. The mean annual incidence of IC varies from 1.6/100 000 (Stewenius, Adnerhill et al. 1995) to 2.4/100 000 (Moum, Ekbom et al. 1997).

In 1970, Kent et al (Kent, Ammon et al. 1970) performed the first retrospective study of clinical and pathological (colectomy) material from 222 patients with fulminant (n = 12) and chronic disease. The aim was to see whether the classical criteria could separate the 2 groups of ulcerative disease of the colon. Fourteen cases (15%) were categorised as "indeterminate" because of "overlapping features" (10%) and "data, insufficient to make a decision" (5%). One out of 12 fulminant cases was classified as indeterminate. "Overlapping features" were described as "severe mucosal and wall involvement."

In 1978, Price et al (Price 1978) went on to examine 30 colectomy specimens which had been designated "colitis indeterminate". Features of the disease included continuous or discontinuous disease with uneven distribution, fissures, nonaggregated transmural inflammation and glandular irregularities. Following retrospective evaluation of pre- and post-operative biopsy samples and the surgical specimens 15 cases remained indeterminate.

In 1979, Lee et al (Lee, Medline et al. 1979) reported on 5 cases of IC in a series of 32 emergency colectomy specimens. Cardinal morphologic findings were extensive ulceration with sharp transition to normal adjacent mucosa and absence of lymphoid aggregates. Fissures reaching the muscularis propria were usually present (Geboes, Colombel et al. 2008).

It was becoming apparent that, primarily in cases of fulminant colitis, a subgroup of resections had been characterised. This group was likely to have severe, often discontinuous, colitis with some degree of rectal sparing grossly. Histologically features included areas without architectural distortion to suggest long-standing disease, deep fissuring ulcers that often went into, and sometimes through, the muscularis propria, accompanied by transmural inflammation, although usually not with the typical lymphoid hyperplasia associated with CD, and without overt granulomas (Geboes, Colombel et al. 2008).

Wells et al (Wells, McMillan et al. 1991) carried out a study of 46 patients who had a colectomy for fulminant colitis. After histological examination of the surgical specimens 16 patients were found to have IC. This group was followed for a median of 10 years, and during this time 12 remained with the diagnosis of IC, 1 was diagnosed with CD and 3 with UC.

Moum et al (Moum, Ekbom et al. 1997) carried out a prospective incidence trial and found that of 36 patients initially diagnosed with IC, 33% had been given a diagnosis of UC and 17% a diagnosis of CD within 1 to 2 years of initial diagnosis.

Meucci et al (Meucci, Bortoli et al. 1999), in their retrospective observational study, showed that the cumulative probability of having a definite diagnosis of either UC or CD was 80% 8 years after initial diagnosis of IC.

Many of the features initially thought to be synonymous with IC, such as fissuring ulceration and transmural inflammation may in fact be those of fulminant colitis of any aetiology. In the accurate classification of fulminant colitis histopathological evaluation alone has its limitations (Guindi, Riddell 2004). Granulomas and transmural lymphoid hyperplasia/aggregates, especially when not in areas of ulceration, appear to be the two most specific indicators of CD in colectomy specimens from patients with fulminant colitis (Swan, Geoghegan et al. 1998). Poorly formed microgranulomas may be good indicators of CD, although when an aggregate of histiocytes becomes a granuloma is highly subjective. A figure of five histiocytes has been used to define microgranulomas in an attempt to provide objectivity (Mahadeva, Martin et al. 2002). Isolated giant cells and well defined epithelioid granulomas distant from crypts do not, as a rule, occur in UC, and hence their presence in a colonoscopic biopsy showing features of chronic IBD is a strong pointer towards the diagnosis of CD. However, crypt associated giant cells and granulomas can occur in UC, and in themselves are unreliable features for the discrimination between CD and UC (Mahadeva, Martin et al. 2002).

It has been hypothesised that treatment with steroids may lead to histological appearances consistent with IC (Rudolph, Uthoff et al. 2002), or that IC simply represents early IBD when the microscopic features required for diagnosis of UC or CD are not yet present (Schumacher, Kollberg et al. 1994). This made be especially relevant in children (Washington, Greenson et al. 2002).

IC in children is more marked than in adults, and can be further complicated by the presence of upper GI disease in patients clinically presenting as UC. Upper GI disease however does tend to resolve, and is far less common in adults (6 – 12%) than in children (20 – 75%) (Geboes, Colombel et al. 2008). During initial diagnosis between 4 and 23% (Auvin, Molinie et al. 2005, Hildebrand, Fredrikzon et al. 1991) of children are classified as having IC. In children diagnosed with IBD under the age of 2 years, 33% are diagnosed with CD, 33% with UC and 33% with IC (Heyman, Kirschner et al. 2005). IC progressively decreases with increasing age, and is present in 9% of IBD patients aged 13 – 17 years (Heyman, Kirschner et al. 2005). Approximately 60% of paediatric patients with IC are reclassified (Bentsen, Moum et al. 2002), more often being UC (Stordal, Jahnsen et al. 2004, Antonioli 2005).

The need to formally classify IC into either CD or UC is very relevant from a medical viewpoint when deciding on the most appropriate from of therapy or entry into clinical trials, and from a surgical standpoint in view of planning the most suitable procedure. It is generally accepted that patients with CD have a poorer outcome than patients with UC when undergoing ileal pouch anal anastomosis (IPAA) due to a high risk of fistula incontinence, pouch failure and anastomotic leaks (Tekkis, Heriot et al. 2005). Reported rates of pouch failure in IC are very variable; Rudolph et al (Rudolph, Uthoff et al. 2002) reported 0% failure rate, McIntyre et al (McIntyre, Pemberton et al. 1995) and Yu et al (Yu, Pemberton et al. 2000), both from the Mayo Clinic, reported rates of 19% and 27% respectively compared to 8% (P=0.03) and 11% ($p< 0.001$) in their UC patients, and Koltum et al (Koltun, Schoetz et al. 1991) reported rates of 50% failure in IC patients compared with 3% in UC patients ($p< 0.001$).

Interesting, Yu et al reported that at ten years of follow-up IC patients had significantly more episodes of pelvic sepsis (17% v 7% ($p<0.001$)) and pouch fistula (31% v 9% ($p<0.001$)) as well as the aforementioned pouch failure. However, during the course of their study 15% of IC patients had their diagnosis revised to one of CD, as did 2% of UC patients. When the outcomes of these patients newly diagnosed with CD were considered separately, the rate of complications for the remaining patients with IC was identical to that of patients with chronic UC. They concluded that after IPAA patients with IC who did not develop CD subsequently experienced long-term outcomes nearly identical to patients with chronic UC. CD, whether it develops after surgery for chronic UC or IC, is associated with poor long-term outcomes.

The variability in outcomes of patients with IC following IPAA may be due to the heterogeneity of the patients included in the IC group of different studies, representing a mixture of CD and UC (Guindi, Riddell 2004). Functional outcomes appear to be similar in both IC and UC patients following IPAA (Rudolph, Uthoff et al. 2002, Pezim, Pemberton et al. 1989, Delaney, Dadvand et al. 2002).

When considering indeterminate colitis we must stress the requirement for new biomarker discovery to minimise diagnositic uncertainty and optimise treatment regimens.

1.6.4 Extraintestinal Manifestations of IBD

In both CD and UC extrainstestinal manifestations of disease are common, occurring in 25-40% of IBD patients (Bernstein, Blanchard et al. 2001b). These can involve nearly any bodily system including musculoskeletal, ocular, dermatological, hepatopancreatobiliary, pulmonary and renal.

Table 1.1: Extraintestinal Manifestations of IBD (Levine, Burakoff 2011)

Site	Manifestation
Musculoskeletal	• Arthritis: colitic type, ankylosing spondylitis, isolated joint involvement • Hypertrophic osteoarthropathy: clubbing, periostitis • Miscellaneous manifestations: osteoporosis, aseptic necrosis, polymyositis
Ocular	• Uveitis/iritis, episcleritis, scleromalacia, corneal ulcers, retinal vascular disease
Hepatopancreatobiliary	• Primary sclerosing cholangitis, cholangiocarcinoma • Associated inflammation: autoimmune chronic active hepatitis, pericholangitis, portal fibrosis, cirrhosis, granulomatous disease • Metabolic manifestations: fatty liver, gallstones associated with ileal Crohn's disease
Dermatological and oral	• Reactive lesions: erythema nodosum, pyoderma gangrenosum, aphthous ulcers, necrotising vasculitis • Specific lesions: fissures, fistulas, oral Crohn's disease, drug rashes • Nutritional deficiencies: acrodermatitis enteropathica, purpura, glossitis, hair loss, brittle nails • Associated diseases: vitiligo, psoriasis, amyloidosis
Metabolic	• Growth retardation in children and adolescents, delayed sexual maturation
Renal	• Calcium oxalate stones

Patients with UC are at higher risk of developing rectal carcinoma, and those with CD small bowel carcinoma and lymphoma. Both UC and CD hold equal risk of the development of hepatobiliary and colon carcinoma (Bernstein, Blanchard et al. 2001a).

1.6.5 Colorectal Cancer and IBD

The inflammatory bowel diseases, both CD and UC, have been associated with an increased risk of developing colorectal cancer (CRC). This is likely due to chronic inflammation promoting carcinogenesis (Ullman, Itzkowitz 2011).

In UC, duration of disease, extensive mucosal involvement, concomitant primary sclerosing cholangitis (Brentnall, Haggitt et al. 1996, Broome, Lindberg et al. 1992, D'Haens, Lashner et al. 1993), a family history of CRC (Nuako, Ahlquist et al. 1998) and early onset of UC (Devroede, Taylor et al. 1971) appear to increase the risk of developing CRC. In 2001 a landmark meta-analysis of over 54 000 patients was published by Eaden et al (Eaden, Abrams et al. 2001). The results suggested that in UC the cumulative risk of colorectal cancer is 2% at 10 years, 8% at 20 years and 18% at 30 years irrespective of the extent of disease. Recently, Castanõ-Milla et al (Castano-Milla, Chaparro et al. 2014) have published an up-to-date meta-analysis, this time encompassing nearly

182000 UC patients. They estimate that the overall incidence rate of colorectal cancer in UC is 1.58 per 1000 patient years, significantly lower than Eaden et al who reported 3 per 1000 patient years back in 2001. Castanõ-Milla et al report an overall incidence rate of 0.91 per 1000 patient years in the first decade after the diagnosis of UC, 4.07 per 1000 patient years in the second decade and 4.55 per 1000 patient years in the third decade. The apparent reduction in colorectal cancer in UC patients may be due to tighter control of inflammation, higher colectomy rates, the use of drugs with chemopreventive effects and a better adherence to endoscopic surveillance programmes in high-risk patients al (Castano-Milla, Chaparro et al. 2014).

The risk of CRC in CD has been investigated less compared to UC. Pooled meta-analyses by Jess et al (Jess, Gamborg et al. 2005) and Lutgens et al (Lutgens, van Oijen et al. 2013) of population-based studies have shown increased risk of CRC in CD when compared to a healthy population, however the standardised incidence ratio was only 1.9 and 1.6 respectively. The risk appears to be higher in those with colonic or rectal involvement (Jess, Gamborg et al. 2005, Lutgens, van Oijen et al. 2013), those with stenosing colonic disease (Lovasz, Lakatos et al. 2013), and in those diagnosed under the age of 30 years (Ekbom, Helmick et al. 1990).

1.7 Treatment of IBD

Due to the chronic relapsing and remitting nature of IBD, the mainstay of treatment involves the initiation of remission and then maintenance of this disease state with medical therapies with the aim of avoiding surgical intervention. Surgery is required to remove diseased segments of bowel, manage intra-abdominal sepsis, and to alleviate intestinal obstruction, fistulation or strictures.

UC can be cured by proctocolectomy. There is no cure for CD and the aim is to maintain remission with medication. Widely utilised treatments are 5-aminosalicylic acid (5-ASA) drugs, corticosteroids, immunosuppressive agents, biological therapies, and antibiotics (Talley, Abreu et al. 2011).

1.7.1 Aminosalicylates

These drugs are believed to act as anti-inflammatory agents as they inhibit nuclear factor kappa B and chemoattractant leukotrienes and alter prostaglandin metabolism (Desreumaux, Ghosh 2006). The bioavailability of 5-ASA preparations is low (Sandborn, Hanauer 2003) and therefore adverse events are rare (Loftus, Kane et al. 2004), however the serious adverse effects include interstitial nephritis, pancreatitis, pneumonitis, pericarditis and hepatitis (Gisbert, Gonzalez-Lama et al. 2007).

1.7.2 Corticosteroids

Corticosteroids inhibit almost every aspect of the immune response (Talley, Abreu et al. 2011). This is through their interaction with glucocorticoid receptors in cell nuclei. They inhibit expression of adhesion molecules and trafficking of inflammatory cells to target tissues including the intestine. They also induce apoptosis of activated lymphocytes and decrease inflammatory cytokine expression (Goulding 2004).

Cortisone in UC was one of the first RCTs (randomised controlled trials) described in modern medicine in 1954 (Truelove, Witts 1954). Since then corticosteroids have been used for acute exacerbations of IBD. They are mainly used as short-term treatment to induce remission rather than a maintenance therapy. Although serious adverse effects such as increased risk of infection (Toruner, Loftus et al. 2008) and psychiatric disorders can occur in short-term use (Brown 2009), the long-term consequences of steroid use, such as loss of bone mineral density and diabetes mellitus, are even more serious (Irving, Gearry et al. 2007).

1.7.3 Immunosuppressant Agents

Thiopurine analogs (6-MP (6-mercaptopurine) and its pro-drug azathioprine), methotrexate, and the calcineurin inhibitors (ciclosporin and tacrolimus) are used in IBD management both as agents to induce and to maintain remission (Talley, Abreu et al. 2011).

The thiopurine analogues are associated with nausea, allergic reactions, acute pancreatitis, hepatitis, risk of infection, malignancy and bone marrow suppression.

The adverse events associated with methotrexate are hepatotoxicity, pneumonitis, infection, malignancy alopecia, stomatitis and myelosuppression.

The main adverse event associated with calcineurin inhibitors is renal toxicity, however hypertension, hirsutism, headache, infection, seizures and paraesthesia can occur (Aberra, Lichtenstein 2005).

Thalidomide has many immundomodulatory properties; inhibiting TNF (tumour necrosis factor), IFN-γ (interferon-γ) and IL-12 (interleukin-12), stimulating IL-4 (interleukin-4) and IL-5 (interleukin-5), and interfering with integrin expression, decreasing circulating helper T cells and inhibiting angiogenesis. It has previously been used in the treatment of IBD, however a recent systematic review has suggested that current evidence is insufficient to support its use to induce or maintain remission in UC or adult CD (Yang, Singh et al. 2015).

1.7.4 Biologicals

The use of anti-tumor necrosis factor alpha antibodies (anti-TNFα) was originally trialed unsuccessfully in the treatment of sepsis (Vilcek 2009). Following this, use in rheumatoid arthritis and IBD has occurred. Since 1998 anti-TNFα has been used IBD in both induction and maintenance of remission (Talley, Abreu et al. 2011).

Infliximab is a chimeric monoclonal antibody to TNFα. It is widely used in the management of moderate to severely active and/or fistulating CD unresponsive to traditional anti-inflammatory agents or a single immunosuppressive agent (Hanauer, Feagan et al. 2002, Sands, Anderson et al. 2004).

Adalimumab and Golimumab are recombinant human antibodies to TNFα and are effective for inducing and maintaining remission in CD and UC (Hanauer, Sandborn et al. 2006, Colombel, Sandborn et al. 2007, Sandborn, Feagan et al. 2014). Both have the advantage over infliximab of subcutaneous administration rather than intravenous, meaning that home administration is standard.

Certolizumab pegol (CDP870) is an anti-TNFα with a unique structure. Unlike Infliximab and Adalimumab, which are based on the human immunoglobulin 1 Fc, certolizumab pegol does not

contain an Fc portion and therefore does not display Fc-mediated effects such as antibody-dependent cell-mediated cytotoxicity, apoptosis or necrosis of neutrophils. Certolizumab pegol is a human monoclonal antibody Fab′ conjugated with polyethylene glycol (PEG), an inert 40-kDa macromolecule used to enhance the pharmacokinetic properties of biologics. This improves the pharmokinetic behaviour of the drug, increasing solubility and stability, and decreasing immunogenicity. Currently certolizumab is not commercially available (Schreiber 2011).

Vedolizumab is a humanized monoclonal antibody. It targets α4β7 integrin, which is expressed in gut-homing lymphocytes. A key ligand for integrin α4β7 is mucosal addressin cell adhesion molecule 1 (MAdCAM-1), which is expressed on the endothelium of venules in the lamina propria of the small intestine and the colon, as well as Peyer's patches. Vedolizumab disrupts the α4β7/MAdCAM-1 interaction, hence blocking a key step in the infiltration of $\alpha4\beta7^{+}$ cells into the gut (Raine 2014). Vedolizumab has been shown to be of most clinical benefit in UC, although some patients with CD will gain benefit (Wang, Zhang et al. 2014). The most common adverse reactions are nasopharyngitis, headache and joint pain.

Patients taking biological therapies are at risk of opportunistic infection (Irving, Gearry et al. 2007), lymphoma (Jones, Loftus 2007), and possibly demyelination disorders (Katsanos, Katsanos 2014).

1.7.5 Antibiotics

Several bacterial species may have a role in the pathogenesis of CD including *Mycobacterium* (Colemont, Pattyn et al. 1988, Feller, Huwiler et al. 2007), *Listeria* and *Escherichia coli* (Hugot, Alberti et al. 2003, Eckburg, Relman 2007, Darfeuille-Michaud, Boudeau et al. 2004). It has been shown that diverting the faecal stream though formation of ileostomy reduces the recurrence of CD in the colon (Janowitz, Croen et al. 1998).

A systematic review of the use of antibiotics has shown that they can induce remission in active UC and prevent relapse in quiescent CD, however, the antibiotics evaluated were diverse and therefore it is not clear which antibiotic should be recommended (Talley, Abreu et al. 2011).

1.8 Risk Factors for IBD

1.8.1 Ethnicity

Ethnicity is known to be a major risk factor for IBD. The Jewish population, especially the Ashkenazi, has been shown to be at significantly increased risk of developing IBD compared with Caucasians (Yang, McElree et al. 1993). In North America, Ashkenazi Jews have a significantly higher incidence of IBD than Sephardic Jews living in North America, indicating a genetic predisposition (Basu, Lopez et al. 2005). However, in Israel, Ashkenazi Jews have a lower incidence than those living in Northern Europe, or North America, suggesting an environmental component (Niv, Abuksis et al. 2000).

Basu et al (Basu, Lopez et al. 2005) discovered significant differences in the subgroups of IBD, as well as serological markers and extraintestinal manifestations within varying racial groups. African and Caucasian Americans were more likely to have CD, whereas Mexican Americans were

predisposed to UC. All Mexican Americans tested with UC were positive for P-ANCA compared to only 40% of Caucasians.

1.8.2 Perinatal and Childhood

The expression of IBD may be influenced by perinatal and childhood events. These include the method of feeding, domestic hygiene and perinatal infections. A Swedish group (Ekbom, Adami et al. 1990) showed that those with a recorded "perinatal health event", classified as infection or serious illness in the mother or child, had a 4-fold increased risk for IBD than a control population. The same study also found those of low socioeconomic status to have a 3-fold increased risk of IBD than controls.

However, the opposite has also been proposed, that a lack of exposure to childhood infections may be a risk factor for IBD, the so called "hygiene hypothosis". This hypothesis proposes that the rising frequency of immunological disorders can be attributed to lack of childhood exposure to enteric pathogens, leading to a greater susceptibility to develop an inappropriate immunological response upon exposure to new antigens later in life (Gent, Hellier et al. 1994, Bernstein, Shanahan 2008, Shanahan, Bernstein 2009).

1.8.3 Breastfeeding

Breastfeeding is known to have protective effects against autoimmune regulated diseases such as bronchial asthma (Gdalevich, Mimouni et al. 2001b), allergic rhinitis (Mimouni Bloch, Mimouni et al. 2002), atopic dermatitis (Gdalevich, Mimouni et al. 2001a) and type I diabetes mellitus (Mamun, O'Callaghan et al. 2014). However, in IBD, whilst some studies have shown a protective association, others have failed to show this effect. A meta-analysis of 17 studies did show a reduction in risk of both CD and UC in those who were breastfed; however the evidence was not of high quality (Klement, Cohen et al. 2004). In a population based case control study, breastfeeding was found to be a risk factor for developing paediatric onset CD (Baron, Turck et al. 2005). Since then a further meta-analysis of the role of breastfeeding in paedatric onset IBD did show protective effects, but again the studies were of poor quality (Barclay, Russell et al. 2009).

In breast fed infants, there are higher concentrations of bifidobacteria and fewer anaerobic bacteria in faeces when compared to bottle fed infants (Fanaro, Chierici et al. 2003). Faecal flora can change up until 2 years of age, demonstrating the potential need for a longer duration of breastfeeding to impact a child's risk of IBD development (Midtvedt, Midtvedt 1992).

1.8.4 Antibiotic Usage

Early and recurrent uses of antibiotics in childhood appear to be a risk factor for the development of IBD (Card, Logan et al. 2004, Shaw, Blanchard et al. 2010, Hviid, Svanstrom et al. 2011, Kronman, Zaoutis et al. 2012).

It is difficult to determine a causal effect as use of antibiotics may alter the gut microbiome but the effect seen may simply be due to the affected individuals being genetically susceptible to infection and thus requiring courses of antibiotics (Ponder, Long 2013).

1.8.5 Non-steroidal Anti-inflammatory Drugs (NSAIDs)

NSAIDs can damage the mucosa of the stomach, small bowel and colon. They also increase intestinal permeability by inhibiting cyclooxygenase (Cipolla, Crema et al. 2002), which reduces prostaglandin production, leading to the inhibition of tumor necrosis factor and the induction of anti-inflammatory cytokines such as interleukin 10 (Berg, Zhang et al. 2002). These factors have been linked to the development of IBD.

A large cohort study showed that regular use of NSAIDs, but not aspirin, was associated with an increased risk of both UC and CD (Ananthakrishnan, Higuchi et al. 2012). Another study showed that NSAID use in those with IBD is associated with frequent and early relapse of quiescent IBD (Takeuchi, Smale et al. 2006).

1.8.6 Smoking

In the early 1980s the first studies were carried out on smoking and IBD. Harries et al noticed that whilst smoking trends in patients with CD were similar to those of the general population, 42% adult males and 37% of adult females of the general population were smokers at the time, those with UC tended to be non-smokers. In their study they showed that 42% of patients with CD and 44% of healthy controls were current cigarette smokers, whereas only 8% of those with UC were ($p<0.001$) (Harries, Baird et al. 1982)**.**

Somerville et al (Somerville, Logan et al. 1984) later studied patients with CD and found that in comparison to a control group those with CD were significantly more likely to be smokers.

In the late 1980s Calkins (Calkins 1989) carried out a meta-analysis and found that smoking and CD, and not smoking and UC and had a consistent association. Current smoking was associated with an OR of 2.0, 95%CI 1.65-2.47 for CD in comparison to non-smokers, and an OR of 1.8, 05%CI 1.33-2.51 in comparison to ex-smokers. The OR for UC in current smokers was 0.41, 95%CI 0.34-0.48 when compared to non-smokers.

Mahid et al (Mahid, Minor et al. 2006) have shown in a recent meta-analysis that their data remain consistent. They showed an association between smoking and CD (OR 1.76, 95%CI 1.4-2.22) and former smoking and UC (OR 1.79, 95%CI 1.37-2.34). They also found that current smoking had a protective effect on the development of UC in comparison to controls (OR 0.58, 95%CI 0.45-0.75).

UC patients that smoke usually have a more benign disease course compared with non-smokers (Lakatos, Szamosi et al. 2007). They have been shown to require hospitalisation for disease flares less often than non-smokers (Boyko, Koepsell et al. 1987), require oral steroids for disease control less often (Mokbel, Carbonnel et al. 1998) and have lower colectomy rates than non-smokers (Mokbel, Carbonnel et al. 1998, Boyko, Perera et al. 1988).

In CD, smoking has been shown to influence disease phenotype. Those who smoke are at increased likelihood of having ileal than colonic or ileocolonic involvement (Lindberg, Jarnerot et al. 1992, Russel, Volovics et al. 1998). They are also more likely to have a disease type that progresses to penetrating, fistulating or stricturing when compared to a non smoking CD cohort (Lindberg, Jarnerot et al. 1992, Picco, Bayless 2003, Louis, Michel et al. 2003). Smokers are also more likely to require immunosuppressive agents (Cosnes, Carbonnel et al. 1999) and surgical resection (Lindberg, Jarnerot et al. 1992, Breuer-Katschinski, Hollander et al. 1996, Sutherland, Ramcharan et al. 1990) than non-smokers. Patients who continue to smoke following surgical resection have been shown to be more likely to develop recurrent disease (Cosnes, Carbonnel et al. 1999, Breuer-Katschinski, Hollander et al. 1996, Sutherland, Ramcharan et al. 1990, Timmer, Sutherland et al. 1998) and to need further surgical intervention (Sutherland, Ramcharan et al. 1990).

In patients with CD receiving anti-TNFα therapy, non-smokers have been shown to experience a higher response rate and a longer duration of response compared to smokers (Parsi, Achkar et al. 2002). It has also been shown that patients who stop smoking have fewer disease flares and require less steroids or immunosuppressants to control their symptoms than those who continue to smoke (Cosnes, Beaugerie et al. 2001).

1.8.6.1 Mechanisms of Action of Smoke

The exact mechanisms by which smoking influences IBD remain unclear. Tobacco had multiple constituents; however it has long been suspected that the metabolite of interest in disease course is nicotine (Birrenbach, Bocker 2004).

Nicotine has been shown to decrease the synthesis of proinflammatory molecules, IL-1β and TNF-α, in murine colonic mucosa as well as the production of mucosal eicosanoids (Motley, Rhodes et al. 1990). It has also been shown to decrease proinflammatory cytokines IL-2 (van Dijk, Meijssen et al. 1998), IL-8 and TNF-α (Wang, Yu et al. 2003) by human mononuclear cells through its action on the nicotinic acetylcholine receptor a7 subunit.

Nicotinic acetylcholine receptors (nAChRs) are present in mucosal epithelial cells of the bowel and are also expressed on T-cells, thus indicating that nicotine may directly influence T-cell function. However, randomised placebo-controlled trials of nicotine patches for active UC have been carried out (Pullan, Rhodes et al. 1994, Sandborn, Tremaine et al. 1997) and although significant clinical improvement was noted in those receiving transdermal nicotine therapy, levels of remission were not shown to be significantly higher in either study. This was confirmed in a study by Thomas et al (Thomas, Rhodes et al. 1995) in which nicotine patches were compared to placebo for maintenance of remission. The nicotine group showed no benefit over placebo. It was also noted by Pullan et al that the side effects from nicotine, headache, nausea and dermatitis, were, in general, greater than the perceived clinical benefits (Pullan, Rhodes et al. 1994).

1.8.6.2 Cytokine Levels

Dysregulated cytokine production is known to be a feature of IBD (Aldhous, Prescott et al. 2008). In CD excess production of IL-1β, IL-12 and TNF-α is noted (Cobrin, Abreu 2005), and in UC, excess IL-13 (Targan, Karp 2005).

Increased levels of IL-4 has been found in smokers (Byron, Varigos et al. 1994), as well as decreased levels of IL-1β, IL-2, IL-8, IL-10, and TNF-α in peripheral blood mononuclear cells, and of IL-1β and IL-8 in colonic tissue of healthy controls and IBD patients who smoke. Sher et al (Sher, Bank et al. 1999) found significantly lower levels of IL-1β and IL-8 in colonic mucosa of smokers with UC when compared to healthy controls, and lower levels of IL-8 in smokers with CD.

Aldhous et al (Aldhous, Prescott et al. 2008) measured cytokine production from peripheral blood mononuclear cells stimulated with lipopolysaccaride (LPS) or phytohemagglutinin (PHA) +/- nicotine in CD and UC patients as well as healthy controls. Following stimulation with LPS and PHA, levels of IL-12/IL-23p40 were significantly higher in IBD patients than healthy controls. PHA stimulation increased IL-1β in UC patients and decreased TGFβ (Transforming growth factor beta) in IBD patients. In both IBD patients and healthy controls, nicotine decreased LPS and PHA induced production of IL-1β, IL-10, TGFβ, and TNF-α. In IBD patients, cell cycle analysis showed that PHA induced proliferation and decreased G_0/G_1 resting cells. Nicotine decreased PHA stimulated S-phase proliferation and increased G_0/G_1 resting cells. They also showed independent associations between IL12/IL23p40 and apoptosis, IL-1β and resting cells, and TNF-α and proliferating cells. They concluded that dysregulated cytokine profiles in CD and UC are associated with specific cell cycle responses and that these effects may be modified by nicotine.

1.8.6.3 Colonic Mucus Production

In UC patients, Pullan et al (Pullan 1996) showed that a significantly thinner mucus layer was present in the colon in both smokers and non-smokers in comparison to healthy controls. This is in contrast to CD, where the mucus layer was shown to be thicker than in controls. A potential cause of this could be the skip lesions in CD facilitating areas with high levels of inflammatory mediators stimulating the release of mucin from adjacent "healthy" goblet cells (Birrenbach, Bocker 2004). Zijlstra et al (Zijlstra, Srivastava et al. 1994) showed that in rabbit rectal mucosa, mucus production is significantly increased with high dose nicotine therapy. Finnie et al (Finnie, Campbell et al. 1996) replicated these results in vitro in both left and right sided colonic epithelial biopsy specimens. Potentially this could explain the seemingly protective effects of nicotine in UC.

1.8.6.4 Tissue Perfusion

IBD patients with acute, severe episodes of inflammation have been shown to have increased gut perfusion, however chronic inflammation in both UC and CD has been shown to result in reduced gut perfusion (Tateishi, Arima et al. 1997, Hulten, Lindhagen et al. 1977, Angerson, Allison et al. 1993). Srivastava et al (Srivastava, Russell et al. 1990) showed that patients with UC have increased blood flow to the rectal mucosa. We know that smoking decreases tissue perfusion and through increased CO (carbon monoxide) concentrations in the serum, may potentiate the impairment in vasodilatation

shown by Hatoum et al (Hatoum, Binion et al. 2003) to occur in chronically inflamed microvessels. This may be beneficial in acute UC but in chronic inflammation the resulting mucosal ischaemia can perpetuate ulceration and fibrosis (Hatoum, Binion et al. 2003).

1.8.7 The Role of Appendicectomy

1.8.7.1 Ulcerative Colitis

In 1987, Gilat et al (Gilat, Hacohen et al. 1987) first reported the inverse relationship between the development of ulcerative colitis and appendicectomy. Their international case-control study of risk factors for IBD in childhood revealed only 3% of UC patients to have had an appendicectomy compared to 10% of control cases.

In the 1990s multiple studies from both the Europe and the USA (United States of America) were in agreement, stating appendicectomy rates in those who went on to develop UC to be 0.6 – 5%, compared to 14 – 25.4% in controls (Rutgeerts, D'Haens et al. 1994, Gent, Hellier et al. 1994, Smithson, Radford-Smith et al. 1995, Minocha, Raczkowski 1997, Parrello, Pavia et al. 1997, Duggan, Usmani et al. 1998).

Koutroubakis et al (Koutroubakis, Vlachonikolis 2000) carried out a meta analysis of case-control studies in 2000 and showed that appendicectomy results in a 69% reduction in the risk of developing ulcerative colitis. These findings have been refined during the study of a large combined cohort of Scandinavian patients (Frisch, Pedersen et al. 2009) which showed a decreased risk of UC in patients who had an appendicectomy, but only if it was for appendicitis or mesenteric adenitis, and only in those < 20 yrs old, rather than in patients who had a negative appendicectomy.

Swidsinski et al (Swidsinski, Dorffel et al. 2011) has shown that acute appendicitis is often caused by *Fusobacterium nucleatum/necrophorum*, and their presence positively correlated with disease severity. It was also evident in their study that the faecal microbiota represented by *Bacteroides, Eubacterium rectale* (*Clostridium group XIVa), Faecalibacterium prausnitzii* groups and *Akkermansia muciniphila* were inversely related to the severity of the appendicitis. The relevance of this finding is that *Faecalibacterium prausnitzii* is known to have anti-inflammatory properties and is also known to be reduced in IBD, and this reduction is associated with CD recurrence after resection (Sokol, Pigneur et al. 2008). Roblin et al (Roblin, Neut et al. 2012) have since postulated that appendiceal dysbiosis may in fact be a trigger in the development of UC, based on previous correlations found between *Fusobacterium*, namely *Fusobacterium varium*, and UC (Ohkusa, Sato et al. 2002), and the improvement of UC symptoms when antibiotic therapy targeted at *Fusobacterium varium* is utilised (Ohkusa, Kato et al. 2010).

1.8.7.2 Crohn's Disease

The relationship between appendicectomy and the development of CD appears more difficult to define. Whilst there are numerous studies stating an increased risk of the development of CD post appendicectomy (Andersson, Olaison et al. 2003, Gearry, Richardson et al. 2010), large cohort studies

(Kaplan, Pedersen et al. 2007) and meta-analysis (Kaplan, Jackson et al. 2008) appear now to refute this phenomenon which may in fact be due to a difficulty in the initial diagnosis of CD.

1.8.8 Measles

During the study of the potential causative relationship between measles and IBD, three main areas have been studied; *in utero* exposure, infection in early life, and laboratory based tests to confirm past or present infection with the virus.

1.8.8.1 *In utero* Infection

Originally, in a Swedish cohort of 25 000 live births, 4 cases of maternal infection with measles virus were identified and 3 of the offspring went on to develop CD (Ekbom, Daszak et al. 1996). Subsequently a Danish study identified 33 cases of maternal measles between 1915 and 1966 and found that of the 26 offspring, 25 were available for follow-up and none had developed CD (Nielsen, Nielsen et al. 1998). A UK based study also showed that maternal exposure to measles did not seem to correlate to cases of CD or IBD in the offspring (Jones, Fine et al. 1997).

1.8.8.2 Early Life Infection

The British Cohort Study carried out by Montgomery et al (Montgomery, Morris et al. 1999) divided early life measles exposure into those infected under the age of 2 years, 2 – 5 years and 6 – 10 years. They found that out of the 20 individuals with CD, none were infected prior to age 2, 5 were infected aged 2 – 5 years (OR 1.06; 95% CI 0.37 to 3.06), and 4 were infected aged 6 – 10 years (OR 1.33; 95% CI 0.42 to 4.19). The risk of UC was not significantly associated with early life measles infection.

An American study (Pardi, Tremaine et al. 2000) followed 662 patients with measles infection under the age of 5 years, for 10 – 48 years. Of these 6 cases of CD and 6 cases of UC were identified, compared with an expected incidence of 1.9 and 2.0 respectively.

In a further British study following two cohorts measles infection under the age of 7 years was not found to be associated with an increased risk of IBD (Thompson, Montgomery et al. 2000).

Haslam et al (Haslam, Mayberry et al. 2000) combined data from 4 registers of patients with CD diagnosed from 1972 – 1989 and found that there was no association between being born in a year of measles epidemics and developing CD.

1.8.8.3 Laboratory Testing of Present or Previous Infection

Methods to confirm, or deny, a link between measles and CD have been extensively studied. It has been shown that hybridisation of measles virus RNA, immunohistochemical staining for measles virus nucleocapsid protein, and electron microscopy identification of antibody labelled measles virus nucleocapsid are more common in CD than in a healthy population (Wakefield, Pittilo et al. 1993, Lewin, Dhillon et al. 1995, Daszak, Purcell et al. 1997). Miyamoto et al (Miyamoto, Tanaka et al. 1995) detected a reaction to a monoclonal antibody derived from measles virus infected cells from

patients with CD and Kawashima et al (Kawashima, Mori et al. 2000) detected measles virus RNA in peripheral mononuclear cells in some patients with CD and UC.

However, these results have failed to be replicated in both intestinal and peripheral blood mononuclear cells using PCR techniques (Iizuka, Nakagomi et al. 1995, Haga, Funakoshi et al. 1996, Afzal, Armitage et al. 1998, Chadwick, Bruce et al. 1998).

1.8.8.4 Measles Vaccine

In 1995, Thompson et al (Thompson, Montgomery et al. 1995) published a cohort study following children vaccinated against measles from 1964 to 1994. The incidence of IBD in this cohort was compared to a cohort of presumably unvaccinated children born in 1958 and showed that the vaccinated group had a 3-fold increased risk of CD and a 2.5- fold increased risk of UC. There were, however, considerable methodological concerns regarding this study (Patriarca, Beeler 1995) but still the possibility of a causal effect had been raised and therefore a number of studies were initiated. Feeney et al (Feeney, Ciegg et al. 1997) showed that there was no association between measles vaccination and CD, UC or IBD as a whole. However, Wakefield et al (Wakefield, Murch et al. 1998) again raised concerns regarding the vaccination by describing non-specific colitis, ileal-lymphoidnodular hyperplasia, and developmental disorders following the vaccination. Thus a large case-controlled study was initiated and no evidence was found the measles vaccine triggered IBD (Davis, Kramarz et al. 2001). This was confirmed by the 1970 British Cohort Study in which no association was found between measles vaccination and IBD (Morris, Montgomery et al. 2000).

Hermon-Taylor et al plotted the course of increase of CD in the UK and found it have initiated 20 years prior to the introduction of the measles vaccine (Hermon-Taylor, Ford et al. 1995), adding to the evidence that the cause of CD or IBD does not stem from the measles vaccine.

It appears that the measles vaccine has been absolved of responsibility as a cause of IBD and in particular CD. The implication of early life measles virus infection however remains open to debate as conflicting evidence exists. This is also the case for the identifiable presence of measles virus in laboratory testing.

1.8.9 The Oral Contraceptive Pill

The oral contraceptive pill (OCP) has been implicated in the development of IBD with a potential mechanism being that the thrombotic properties of oestrogen exposure lead to a process of multifocal, microvascular gastrointestinal infarction (Wakefield, Sawyerr et al. 1991). A meta-analysis of over 75 000 women calculated that the pooled relative risk (RR) for the development of CD in women taking the OCP was 1.51, and 1.46 when adjusted for smoking. The RR for UC in women taking the OCP was 1.53, and 1.28 when adjusted for smoking (Cornish, Tan et al. 2008).

More recently a prospective cohort study compared users of the OCP with those who had never used the OCP. The multivariate-adjusted hazard ratios for CD were 2.82 (95% CI 1.65 to 4.82) among current users and 1.39 (95% CI 1.05 to 1.85) among past users. The association between OCP use and UC was limited to women with a history of smoking (Khalili, Higuchi et al. 2013).

1.8.10 Diet

It has been hypothesised that diet may have a role to play in the development of IBD. There has been an increase in the development of IBD in countries where it was previously rare, in parallel with the adoption of a western diet (Thia, Loftus et al. 2008). Increasing intake of dietary fat, particularly n-6 polyunsaturated fatty acids (PUFAs) and reduced intake of n-3 PUFAs is common in the Western diet. Through the arachidonic acid pathway, dietary n-3 PUFA competitively inhibits the formation of proinflammatory prostaglandins and leukotrienes (Marion-Letellier, Savoye et al. 2013). It also inhibits vascular adhesion and migration, angiogenesis, and adaptive immune responses through peroxisome proliferator-activated receptor γ (PPARγ) and nuclear factor κB (NFκB) mediated pathways (Hjerkinn, Seljeflot et al. 2005, Yang, Lu et al. 2012, Calder 2012).

A large prospective study of over 170 000 women concluded that high intake of dietary long-chain n-3 PUFAs may be associated with a reduced risk of UC (hazard ratio 0.72, 95% CI 0.51 to 1.01). However, high long-term intake of trans-unsaturated fats may be associated with a trend towards an increased incidence of UC (hazard ratio 1.34, 95% CI 0.94 to 1.92) (Ananthakrishnan, Khalili et al. 2014). The same group also found that long-term intake of dietary fibre, particularly from fruit, is associated with lower risk of CD but not UC (Ananthakrishnan, Khalili et al. 2013).

A recent literature review by Andersen et al (Andersen, Olsen et al. 2012) found that prospective studies suggest that a diet high in protein, particular animal protein, may be associated with increased risk of IBD and a higer risk of disease relapse, and that n-6 PUFAs may predispose to UC whilst a diet high in n-3 PUFA may protect from UC. These findings however were not replicated in the review by Spooren et al (Spooren, Pierik et al. 2013). They stated that although some studies report high intake of sugars and low intake of fruit and / or vegetables to be associated with increased risk of IBD, this cannot be confirmed in other studies and thus the evidence is lacking. Currently the GEM Project (www.gemproject.ca) and the PREdiCCT Study are recruiting to investigate causes of IBD.

1.9 Areas of Research Interest in IBD

Over the past two decades, the majority of IBD research has been focused around microbiological, immunological and genetic studies. Thus I have chosen to focus on these areas of research interest whilst reviewing our current knowledge.

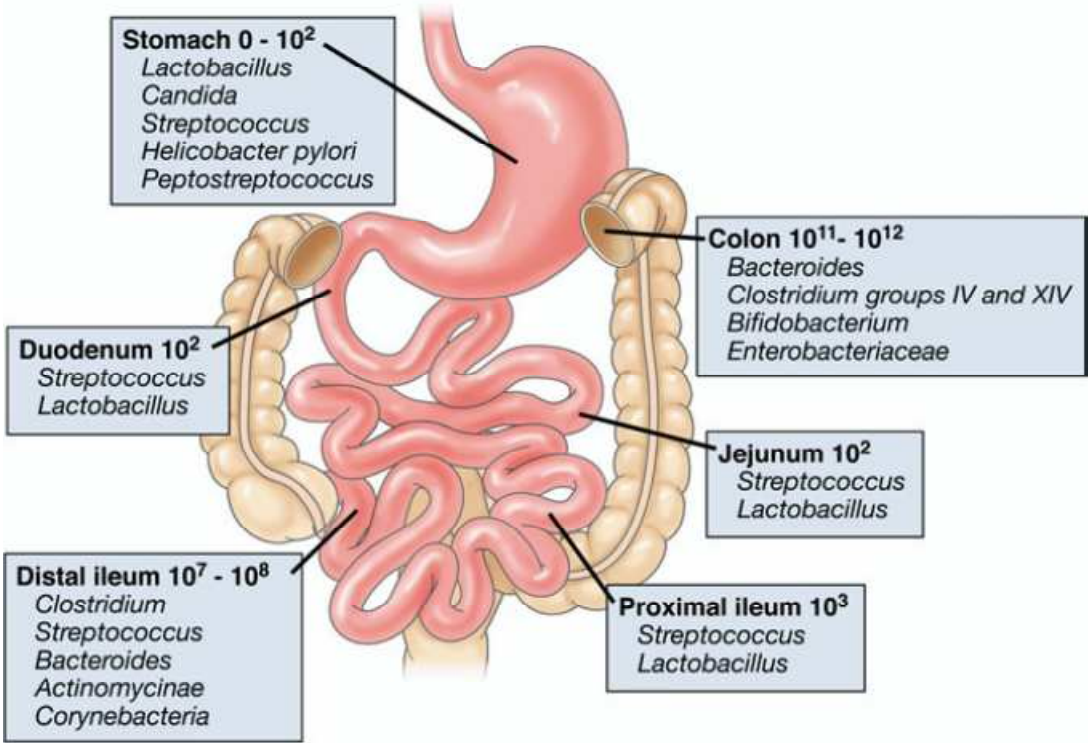
1.9.1 Microbes in IBD

At birth the human gut is sterile; however, bacterial colonisation begins within a few hours of life. Bacterial diversity increases rapidly in infancy, initially based on the method of birth and infant feeding (Penders, Thijs et al. 2006), eventually resulting in a distinct but relatively stable adult gut microbiome (Dominguez-Bello, Blaser et al. 2011).

It is estimated that 10^{14} individual bacteria (Gill, Pop et al. 2006, Davis 1996) from >1000 species reside in the human gut, and that their genome is 150 times that of the human genome (Qin, Li et al.

2010). Within the human gut the majority of bacteria (98%) belong to four phyla; *Firmicutes* (64%), *Bacteroidetes* (23%), *Proteobacteria* (8%), and *Actinobacteria* (3%) (Frank, St Amand et al. 2007).

Figure 1.3: Microbiota of the GI tract (Sartor 2008)



In healthy people, the mucosal microbiome appears to form a stable ecosystem with the host immune system, whereas the luminal and faecal microbiome may alter with environment and diet (Eckburg, Bik et al. 2005).

Current thinking denotes that in order for a chronic inflammatory reaction to be initiated, the host must either fail to identify and respond to pathogenic bacteria on initial contact, or must fail to initiate autophagic mechanisms of intracellular bacterial killing (Mukhopadhya, Hansen et al. 2012). Thus either the NOD2 (Nucleotide-binding Oligomerisation Domain-containing Protein 2) pathway or the ATG16L1 (Autophagy-related 16 Like 1) pathway is defective.

Single bacteria have been studied in order to attempt to identify a causative agent for IBD. *Mycobacterium avium* subspecies *paratuberculosis* (MAP) has been extensively studied in both humans and in animal species; however despite a couple of meta-analyses recently published, a pathogenic effect has not been identified in CD (Feller, Huwiler et al. 2007, Abubakar, Myhill et al. 2008).

IBD patients appear to have a gut microbiome different in composition to healthy individuals. Studies have shown 10 – 100 fold decreases in abundances of bacteria beneficial to the host such as *Firmicutes,* specifically *Faecalibacterium prausnitzii* and *Bacteroidetes,* and an increase in the *Gammaproteobacteria* (Frank, St Amand et al. 2007, Sokol, Seksik et al. 2009, Frank, Robertson et al.

2011, Morgan, Tickle et al. 2012). It remains unclear, however, whether this dysbiosis in IBD is an effect of the disease or whether it is a contributory factor.

Table 1.2: Alterations in Microbial Composition and Function Linked to IBD (Kostic, Xavier et al. 2014)

Microbial composition	Decrease in α diversity Decrease in *Bacteroides* and *Firmicutes* Increase in *Gammaproteobacteria* Presence of *E coli*, specifically adherent invasive *E coli* Prescence of *Fusobacterium* Decrease in *Clostridia*, *Ruminococcaceae*, *Bifidobacterium*, *Lactobacillus* Decrease in *F prausnitzii*
Microbial function	Decrease in SCFAs, butyrate Decrease in butanoate and propanoate metabolism Decrease in amino acid biosynthesis Increase in auxotrophy Increase in amino acid transport Increase in sulfate transport Increased oxidative stress Increase in type II secretion systems, secretion of toxins

In CD, proportions of the *Clostridia* are altered: the *Roseburia* and *Faecalibacterium* genera of the *Lacchnospiracae* and *Ruminococcaceae* families are decreased, whereas *Ruminococcus gnavus* is increased (Sokol, Seksik et al. 2009, Joossens, Huys et al. 2011, Sokol, Pigneur et al. 2008).

Dysbiosis in UC is less well described, although increased sulphate-reducing *Deltaproteobacteria* have been reported (Gibson, Cummings et al. 1991, Roediger, Moore et al. 1997).

Bacterial genes greater than 100 times that of the human gene set encode the normal gut microbiome (Eckburg, Bik et al. 2005, Qin, Li et al. 2010). Therefore the potential for treatments, diets and medications to affect the microbiome as well as the human host is great. Dietary fibre, the main energy source of the lower gastrointestinal tract microbiotia, is a prime example (Flint, Bayer et al. 2008). Fibrolytic bacteria degrade polysaccharides into smaller carbohydrates, which are then fermented into short-chain fatty acids (SCFAs) such as acetate, propionate and butyrate. All of these have immunomodulatory properties, and butyrate, in particular, is a major source of energy for colonocytes (Tedelind, Westberg et al. 2007, Clausen, Mortensen 1995, Inan, Rasoulpour et al. 2000, Segain, Raingeard de la Bletiere et al. 2000, Saemann, Bohmig et al. 2000, Velazquez, Lederer et al. 1997).

Genetic studies in IBD pathogenesis have highlighted the role of host-microbe interactions (Khor, Gardet et al. 2011, Barrett, Hansoul et al. 2008, Franke, McGovern et al. 2010, McGovern, Gardet et

al. 2010), specifically the microbial responses in IBD of T-cell activation, the IL-23/T helper 17 pathway, autophagy (Hampe, Franke et al. 2007), and Paneth cell function (Vaishnava, Behrendt et al. 2008). This supports the theory of host-microbiotia interface for gut homeostasis and also for dysfunctional interactions between host and gut microbiome in IBD.

Morgan et al (Morgan, Tickle et al. 2012) analysed the gut microbiome of CD and UC patients and healthy controls using multivariate metagenomic analysis and taking into account environmental factors such as treatments, age and smoking in order to measure compositional and functional differences in gut microbiota. Age was associated with a decrease in *Bifidobacterium*, as shown in previous studies (Agans, Rigsbee et al. 2011, Mariat, Firmesse et al. 2009), and disease activity was not independently associated with a specific shift in the microbiome composition. There was no significant association between microbiome composition and gender.

Analysis of the microbiome showed *Roseburia* and *Phascolarctobacterium* to be significantly reduced in both UC and CD, whilst *Clostridium* was increased. *Roseburia*, which utilises acetate and produces butyrate (Duncan, Hold et al. 2002), is associated with anti-inflammatory T cell production in the gut (Atarashi, Tanoue et al. 2011). *Phascolarctobacterium* are succinate consumers, producing propionate when co-cultured with *Paraprevotella* (Watanabe, Nagai et al. 2012). Thus the decreases in *Roseburia* and *Phascolarctobacterium* seen in IBD may reflect a decrease in butyrate and propionate production.

In CD, the *Ruminococcaceae*, which produce acetate (Chassard, Bernalier-Donadille 2006), were decreased, and in UC the *Leuconostocaceae*, which produce acetate and lactate (Cogan, Jordan 1994), were decreased. In CD, the *Enterobacteriaceae*, specifically *Escherichia / Shigella*, which have previously been associated with intestinal inflammation (Baumgart, Dogan et al. 2007, Garrett, Gallini et al. 2010, Kleessen, Kroesen et al. 2002, Mylonaki, Rayment et al. 2005), were increased.

Ileal CD was studied as a distinct microbiome phenotype. The *Ruminococcaceae* family was found to be dramatically reduced, especially *Faecalibacterium*. *Faecalibacterium prausnitzii* is able to metabolise diet-derived polysaccarides and host-derived substrates such as N-acetyl glucosamine from intestinal mucous (Lopez-Siles, Khan et al. 2012). It is also a major butyrate producer and inhibits anti-inflammatory effects in the setting of colitis (Sokol, Pigneur et al. 2008). The *Ruminococcaceae* consume hydrogen and produce acetate that can be utilised by *Roseburia* produce butyrate (Duncan, Hold et al. 2002, Chassard, Bernalier-Donadille 2006). A reduction of these organisms may have functional consequences on the ability of the host to repair the epithelium and regulate inflammation.

1.9.1.1 The Faecal Microbiome

Human infants have very heterogeneous, unstable and distinctive microbiota, whereas weaned children and adults show high functional uniformity (Kurokawa, Itoh et al. 2007, Palmer, Bik et al. 2007). It is important to note that analysis of the microbiome based on faecal samples varies from that based on gut biopsies (Morgan, Tickle et al. 2012).

Microbes in the human gut undergo selective pressure from the host as well as from microbial competitors leading to homeostasis of the ecosystem. Arumugam et al (Arumugam, Raes et al. 2011) identified three enterotypes, based on faecal samples, by variation in the levels of one of the three

genera: *Bacteroides* (enterotype 1), *Prevotella* (enterotype 2) and *Ruminococcus* (enterotype 3). The drivers of enterotype 1, enriched in *Bacteroides* and co-existing with *Parabacteroides*, derive energy primarily from carbohydrates and proteins through fermentation since these closely related genera have a broad saccharolytic potential and since genes encoding enzymes involved in the degradation of these substrates (galactosidases, hexosaminidases, proteases) along with glycolysis and pentose phosphate pathways are enriched in this enterotype.

Enterotype 2, enriched in *Prevotella* and co-existing with *Desulfovibrio*, can act in synergy to degrade mucin glycoproteins present in the mucosal layer of the gut.

Enetrotype 3, the most common enterotype, is enriched in *Ruminococcus* and *Akkermansia*. Both are known to comprise species able to degrade mucins and are enriched in membrane transporters of sugars, suggesting the efficient binding of mucin and its subsequent hydrolysis as well as uptake of simple sugars by these genera.

As well as conversion of complex carbohydrates into absorbable substrates, the gut microbiotia produces vitamins. Enterotypes 1 and 2 were enriched in biosynthesis of different vitamins: biotin, riboflavin, pantothenate and ascorbate in the former and thiamine and folate in the latter.

With the exception of enterotype 1, which is most common in Japanese individuals, enterotypes do not seem to correlate with nationality, gender, age or BMI (body mass index). It may be however, that future analyses will allow enterotyping of individuals to determine responses to diet or medication strategies.

1.9.1.2 *E.coli* and IBD

Escherichia coli colonisation has been described in IBD patients (Mylonaki, Rayment et al. 2005, Conte, Schippa et al. 2006) and in abundance in those having disease flares (Darfeuille-Michaud, Neut et al. 1998, Cooke, Ewins et al. 1974). *E. coli* strains are classified into four phylohenetic groups, A, B1, B2, and D. Groups A and B1 are commensal strains and carry few virulence genes. Groups B2 and D usually possess virulence genes which favours persistence, adhesion and extraintestinal infection (Johnson, Delavari et al. 2001).

In 2007, Kotlowski et al (Kotlowski, Bernstein et al. 2007) described a link between *E. coli* of the phylogenetic groups B2 and D, and IBD. *E. coli* of the serotype most commonly associated with urinary tract infections will often be found within phylogenetic group B2 whereas commensal faecal *E. coli* will not (Navidinia, Peerayeh et al. 2014). A recent meta-analysis (Petersen, Halkjaer et al. 2015) has confirmed the relationship between group B2 *E. coli* colonisation and IBD. It was mainly in UC patients that this relationship was statistically significant, and these samples were from gut biopsies in the majority of cases rather than faecal cultures. The relationship between group D *E. coli* and IBD could not be confirmed as statistically significant in this study.

E. coli is thought to be a trigger in the pathogenesis of IBD through toll-like receptor signalling (Cario 2010). Lipopolysaccharides are produced by these gram-negative bacteria, which, in turn activate toll-like receptor 4 (TLR4) signalling and cause intestinal inflammation through activation of two signalling pathways: the early myeloid differentiation primary response gene 88 (MyD88)-dependent,

and the delayed MyD88-independent response (Zughaier, Zimmer et al. 2005). The MyD88-dependent cascade includes activation of the NF-κB pathway, involving recruitment of the IL-1R-associated kinases (IRAKs), phosphorylation of IκB kinase (IKK) and subsequent phosphorylation and degradation of the family of IκB proteins, which allow phosphorylation of NF-κB followed by its translocation into the nucleus and transcription of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6 and IL-8 (Zeytun, Chaudhary et al. 2010, O'Neill, Bowie 2007, Haddad, Abdel-Karim 2011, Medvedev, Kopydlowski et al. 2000).

Toll-like Receptor 4 (TLR4) has been shown to be strongly upregulated in both CD and UC (Cario, Podolsky 2000).

1.9.1.2.1 Adherent Invasive *E. Coli*

Adhesion allows bacteria to colonise the mucosa and resist mechanical removal from the intestine.

Adhesive *E. coli* have been isolated from 68% of patients with UC, 62% of CD patients and only 6% of healthy controls (Giaffer, Holdsworth et al. 1992). More recently, Kotlowski et al (Kotlowski, Bernstein et al. 2007) reported adhesive *E. coli* were more likely to be associated with UC and CD tissues than controls.

CD-associated *E. coli* preferentially adheres to differentiated Caco-2 cells, which represent a mature intestinal cell model (Rolhion, Darfeuille-Michaud 2007). This is consistent with crypt epithelial cells rarely being involved in early CD lesions (Sankey, Dhillon et al. 1993).

Early CD lesions mainly occur in Peyer's patches (Fujimura, Kamoi et al. 1996), induced by entero-invasive pathogens, such as *Shigella* and *Salmonella*. The aphthous ulcer, the earliest lesion of CD, is a necrosis of M-cells of Peyer's lymphoid follicles (Rickert, Carter 1980). The presence of intramucosal *E. coli*, or mucosa-associated *E. coli* with invasive properties has been reported in 29 – 36% of CD patients, 12 – 19% of UC patients and only 3 – 9% of healthy controls (Martin, Campbell et al. 2004, Darfeuille-Michaud, Boudeau et al. 2004, Alpern, Sasaki et al. 2006). Strain LF82 invades multiple human epithelial cell lines including Hep-2 cells, and the intestinal cell lines Intestine-407, Caco-2 and HCT-8 (Boudeau, Glasser et al. 1999). The uptake of LF82, and of the invasive *E. coli* strains isolated from CD patients is dependent on both functioning host cell actin monofilaments and microtubules (Boudeau, Glasser et al. 1999).

Adherent Invasive *E. coli* (AIEC) are a pathogenic group of *E. coli.* They have the ability to adhere to and invade intestinal epithelial cells with a macropinocytosis-like process of entry dependent on actin microfilaments and microtubules recruitment. They also have the ability to survive and replicate extensively in large vacuoles within macrophages without triggering host cell death, and the ability to induce the release of large amounts of TNF-α by infected macrophages (Rolhion, Darfeuille-Michaud 2007).

AIEC strains have been found to be highly associated with ileal mucosa in CD patients being found in 36.4% of ileal specimens from CD patients, compared to 6% of healthy controls. In colonic specimens

AIEC was identified in 3.7% of CD patients, 0% of UC patients and 1.9% of healthy controls (Darfeuille-Michaud, Boudeau et al. 2004).

Genetic studies have identified mutations in the NOD2/CARD15 encoding gene associated with the severity of ileal CD (Hugot, Chamaillard et al. 2001, Ogura, Bonen et al. 2001). The expression of functional NOD2 may play a role in the clearance of bacteria, even AIEC. Intestinal epithelial cells that do not express NOD2, or that express NOD2/CARD15 3020insC CD-associated variant, are unable to contain intracellular bacterial replication (Kobayashi, Chamaillard et al. 2005, Hisamatsu, Suzuki et al. 2003). Thus ileal CD could result from dysfunction of the NOD2 innate immune surveillance mechanism (Rolhion, Darfeuille-Michaud 2007).

1.9.1.3 Ulcerative Colitis Microbiome

Ulcerative colitis patients express lower biodiversity than healthy controls. In pancolitis patients Morgan et al (Morgan, Tickle et al. 2012) reported a reduced abundance of *Odoribacter* genus, which belongs to the *Porphyromonadaceae* family and the *Bacteroides* phylum. *Odoribacter splanchnus* produces acetate, propionate and butyrate (Goker, Gronow et al. 2011), thus decreased *Odoribacter* may reduce SCFA availability and affect host inflammatory responses.

1.9.1.3.1 Sulphate-reducing Bacteria in Ulcerative Colitis

Sulphate-reducing bacteria (SRB) are Gram-negative, non-spore-forming, obligate anaerobes (Rowan, Docherty et al. 2009). SRB involved in bowel colonisation are flagellate *Vibrio* bacteria. They can travel within bioflims such as the mucous layer of the colon (Devereux, Delaney et al. 1989). *Helicobacter pylori* is the equivalent in the stomach (Rowan, Docherty et al. 2009).

SRB scavenge hydrogen and short-chain fatty acids with high affinity. They produce hydrogen sulphide (Pfennig, Widdel 1982). Hydrogen sulphide is freely permeable to the cell membrane. It does not have specific cell receptors and is known to affect adenosine 5'-triphosphate-dependent potassium channels, DNA integrity, and cytochrome *c* oxidase and carbonic anhydrase activity (Szabo 2007).

Hydrgogen sulphide has been shown to impede butyrate oxidation in colonocytes (Roediger, Duncan et al. 1993). Colonisation with hydrogen-sulphide producing SRB, failed homeostasis of endogenous hydrogen sulphide production, and diminished clearance of excessive exogenous hydrogen sulphide are potential pathogenic pathways in UC (Rowan, Docherty et al. 2009).

1.9.1.4 Amino Acid Biosynthesis and Carbohydrate Metabolism

As described above, the basic gastrointestinal microbiome is altered in both CD and UC. Genes for the metabolism and biosynthesis of most amino acids, especially histine and lysine, are decreased, while arginine, histadine, and lysine transport genes are increased.

In ileal CD, there is a decrease in gluatmine-related functional models. This leads to a lower amount of glutamate that is required for gamma-aminobutyric acid, ornithine, and arginine biosynthesis. Therefore, levels of these molecules are reduced.

Genes for the metabolism of the sulphur-containing amino acid cysteine are significantly increased in IBD, especially in ileal CD. This corresponds to an overrepresentation of genes related to sulphate transport in both UC and CD, and an increase in sulphur and nitrogen metabolism in CD.

CD is associated with an increased abundance of many genes related to carbohydrate transport. In ileal CD, there are increases in the pentose phosphate pathway and an abundance of the fructose / mannose metabolism gene. There is also an increased abundance of transporter genes for glucose, hexoses, maltose and mono-, di-, and oligosaccharides, whereas butanoate and propanoate metabolism is decreased. The latter may be a result of decreased levels of *Roseburia* and *Faecalibacterium* resulting in decreased SCFA production (Morgan, Tickle et al. 2012).

Glutathione transport gene abundance is increased in CD and UC, and in UC there is an increase in glutathione metabolism gene abundance. Glutathione, a tripeptide of cysteine and glutamate, is synthesised by *Proteobacteria* and a few *streptococci* and *enterococci* (Sherrill, Fahey 1998). It allows bacteria to maintain homeostasis during oxidative or acid stress. Inflammatory cascades include the production of highly reactive oxygen and nitrogen metabolites. These are greatly increased in IBD (Keshavarzian, Banan et al. 2003).

During inflammation, lamina propria monocytes release homocysteine, contributing to oxidative stress. High levels of both mucosal and serum homocysteine have been identified in IBD (Danese, Sgambato et al. 2005).

The increases in sulphate transport, cysteine metabolism and glutathione metabolism may reflect gut microbiome mechanisms addressing the oxidative stress caused by inflammation (Morgan, Tickle et al. 2012).

1.9.1.5 Microorganisms as Pathogens in IBD

Both UC and CD result from the inappropriate activation of intestinal mucosa immunity in genetically susceptible hosts. However, increasingly intestinal microbiota are deemed to have a role in the initiation, maintainance and phenotypical expression of IBD (Sartor 2008, Sartor 2010).

Two hypotheses exist in relation to microbial pathogenesis of IBD:

- Specific microorganisms have a role in induction.
- Metabolites derived from the microbiotia can be medicators of injury and inflammation in a perpetuating inflammatory cycle.

1.9.1.5.1 Microorganisms Linked to IBD Pathogenesis

Multiple organisms have been linked to IBD including *Mycobacterium paratuberculosis*, *Listeria monocytogenes,* paramyxovirus, *Chlamydia trachmatis, Shigella, Salmonella, Yersinia, Saccharomyces cerevisiae, Escherichia coli, Eubacteria, Pertostreptococcus, Coprococcus,* and *Bacteriodes vulgatus* (Linskens, Huijsdens et al. 2001).

I have concentrated on *Mycobacterium paratuberculosis,* which causes Johne's disease, a granulomatous infectious ileitis in ruminants. This is very similar to ileal Crohn's disease in humans and this hypothesis was first published in 1913 (Dalziel 1913). *M. paratuberculosis* has therefore been

investigated as a cause of CD, and was first cultured from the intestinal tissue of patients with Crohn's disease by Chiodini et al (Chiodini, Van Kruiningen et al. 1984). However, there is now conflicting evidence with studies reporting detection rates of between 0 and 100% in CD patients (Frank, St Amand et al. 2007, Sartor 2005). In addition, some clinical studies of anti-mycobacterial therapy have failed to show a sustained response, suggesting that this pathogen is not involved in the initiation or progression of CD (Selby, Pavli et al. 2007, Thomas, Swift et al. 1998). However, evidence is slightly conflicted as a systematic review identified anti-mycobacterial therapy to be useful in the maintainence of remission when given with preceding corticosteroid therapy to induce remission (Borgaonkar, MacIntosh et al. 2000).

1.9.1.5.2 Microbiotia Metabolic Mediators of Intestinal Injury or Inflammation

The production of certain microbial metabolites may be mediators of mucosal injury.

Significant alterations in microbial function have been identified between inflamed and non-inflammed regions of UC patients, in comparison with CD patients. This is consistent with the theory that UC is a regionalised disease, whereas CD is a more systemic disease. In inflamed regions of UC patients, the metabolic activities of adherent bacteria have a reduced abundance of genes utilised for carbohydate and nucleotide metabolism, and an increased abundance of genes for lipid and amino acid metabolism. Bacteria that are highly dependent on nutrients from the host environment may have a reduced genome, lacking in genes specific for metabolic pathways (Davenport, Poles et al. 2014). In inflamed tissue, fewer carbohydatres are available, and therefore bacteria that are able to undergo amino acid and lipid metabolism dominate.

Anaerobic degradation of undigested or endogenous protein in the colon results in the production of a wide range of metabolites that are in direct contact with the colonic mucosa. Products of protein fermatation such as ammonia, phenolic compounds and tryptophan metabolites have been found to be potentially carcinogenic, and thus high dietary meat intake has been linked to colorectal cancer (Chao, Thun et al. 2005).

In the colon, protein degradation commences with hydrolysis of proteins to smaller peptides and amino acids by bacterial proteases and peptidases that are more active at neutral to alkaline pH. In the proximal colon the pH is more acidic due to the production of SCFAs from carbohydrate fermentation. Moving distally in the colon carbohydates levels become depleted, pH increases and protein fermentation becomes more efficient (Windey, De Preter et al. 2012).

SCFAs are the major end product from carbohydate fermentation but they are also produced from many amino acids by reductive deamination. SCFAs are beneficial to the host and are rapidly absorbed from the colon. Butyrate is the most important energy source for colonocytes and plays a major role in proliferation and differentiation (Lupton 2004). Butyrate also inhibits colonic carcinogenesis and inflammation, reduces oxidative stress and reinforces the colonic defense barrier (Hamer, Jonkers et al. 2008). Lack of SCFAs in UC stimulated the use of SCFAs as therapy in left sided UC. Recently the use of oral butyrate in DSS induced colitis in mice has shown promising

results in improving mucosal lesions and decreasing inflammatory profiles (Vieira, Leonel et al. 2012).

Branched chain fatty acids (BCFA) exclusively originate from fermentation of branched amino acids. Isobutyrate, isovalerate and 2-methylbutyrate are produced from the fermentation valine, leucine and isoleucine respectively (Smith, Macfarlane 1997). Increased levels of BCFAs signify limited carbohydrate levels.

Ammonia is produced by bacteria through deamination of amino acids and through urea hydrolysis, catalysed by bacterial urease activity. Ammonia can be utilised by bacteria in protein synthesis, or absorbed by colonocytes, metabolised in the liver and is excreted in the urine as urea (Blachier, Mariotti et al. 2007).

Phenolic and indolic compounds are produced from the bacterial degradation of aromatic amino acids. BCFAs, phenols and indoles are unique colonic bacterial metabolites and therefore can be considered markers of protein fermentation in the colon (Geypens, Claus et al. 1997). >90% of urinary phenolic compounds are excreted as *p*-cresol (Hughes, Magee et al. 2000).

Exposure of human colonic epithelial cells to phenol reduces viability. Transepithelial resistance of Caco-2 cells is decreased after incubation with both phenol and also ammonia (Pedersen, Brynskov et al. 2002, Hughes, Kurth et al. 2008, McCall, Betanzos et al. 2009). Permeability of endothelial cells is increased after exposure to *p*-cresol (Cerini, Dou et al. 2004).Thinning, or increased permeability of the mucous layer, is likely to increase the accessibility of potentially damaging agents to the colonic mucosa (Windey, De Preter et al. 2012).

Fermentation of dietary and mucinous sulphate and sulphur amino acids such as methionine, cystine, cysteine and taurine by SRB leads to the production of hydrogen sulphide (H_2S) (Lewis, Cochrane 2007, Roediger, Moore et al. 1997).

H_2S is toxic to colonic cells. In human intestinal epithelial cells, the expression of genes involved in cell-cycle progression, inflammation and DNA repair response is modulated by sulphide (Attene-Ramos, Nava et al. 2010). Sulphide also prevents the oxidation of butyrate in colonocytes, inducing an energy deficient state ultimately resulting in reduction of absorption of sodium, reduction of the secretion of mucin, and a reduced life span of colonocytes (Roediger, Moore et al. 1997). Cellular respiration is also inhibited by H_2S. It acts as an inhibitor of cytochrome c oxidase, the final step in the production adenosine triphosphate (Medani, Collins et al. 2011).

Decarboxylation of amino acids results in amines in the gut. Monoamine and diamine oxidases detoxify the amines produced by gut microbiota. Amines also have a role in the formation of *N*-nitrosamines by condensation of a secondary amine with nitrite in an acidic environment or at neutral pH, when catalysed by bacterial enzymes (Tricker 1997).

Bacterial sulphatase has a critical role in determining the rate of degradation of secreted mucous. There is significantly increased faecal mucin sulphatase activity in patients with UC, compared to healthy controls. CD patients also have increased levels but these are not significant. In UC, faecal sulphatase activities were highest in those with active disease. It is hypothesised that the increase in faecal sulphatase activity contributes to the colitic process by increasing the mucin degradation and

therefore upsetting homestasis between mucous production and bacterial degradation, rather than an alteration in faecal flora causing increased faecal sulphatase activity (Tsai, Dwarakanath et al. 1995).

The human microbiome remains an area requiring a great deal of research to aid our understanding and guide our knowledge with regards to IBD pathogenesis. It is likely that high throughput technologies, such as metabolomics, will greatly advance this field. Hopefully we will gain knowledge of a healthy metabolomic profile in relation to intrinsic properties such as human genetics, diurnal cycles, and age, and extrinsic factors such as diet, pharmacology and habitat (Goodacre 2007). We can then begin to assess the alterations manifested in the metabolite complement in relation to changes in the microbiome seen in diseases such as IBD.

1.9.2 Gut Immunology

The immune system has a major role to play in IBD pathogenesis. In intestinal health, there is symbiosis between the host and the gut microbiome maintained by autoregulatory pathways. However in IBD, dysregulation of this system occurs and chronic inflammation ensues.

To provide defense against infection in the gut, secondary lympoid tissues and immune-system cells are present throughout the gut. Gut-associated lymphoid tissues (GALT) comprise two functional compartments: the inductive compartment and the effector compartment.

The inductive compartment is where interactions between antigen, dendritic cells and lymphocytes induce adaptive immune resposes. The effector compartment, comprised of the lamina propria, is where plasma cells, effector T cells, macrophages, mast cells and eosinophils reside (Parham 2015).

In normal healthy intestine, the mucosal barrier comprises:

- The mucous layer
- The glycocalyx, a filamentous layer of branched carbohydrates
- A layer of epithelial cells held together by tight junctions

1.9.2.1 Mucous Layer

The small bowel has a discontinuous layer of mucous. The colon has a continuous mucous layer comprised of two layers, the upper “sloppy” layer, and the lower adherent layer (Johansson, Phillipson et al. 2008, Johansson, Larsson et al. 2011). The physical properties of mucous are due to secreted mucins, or mucous glycoproteins. In both the small and large bowel, the MUC2 mucin, a large complex glycoprotein, predominates. MUC2 contains a protein core rich in proline, serine and threonine (Backstrom, Ambort et al. 2013, Johansson, Ambort et al. 2011). Other mucins (MUC3, MUC12, MUC13) attach to the epithelial layer via a cytoplasmic tail. This layer acts as a lubricant and also a sieve-like barrier, preventing pathogens accessing the epithelium (Merga, Campbell et al. 2014).

1.9.2.2 Glycocalyx

The glycocalyx is a layer of filamentous material coprised of glycoproteins and glycolipids integrated into the cell membrane. It has long branched polysaccaride chains forming a network covering the

epithelial layer. This layer is thinnest over the surface of M (microfold) cells. The glycocalyx provides a barrier to enteric pathogens, and a surface for the attachment of commensal bacteria (Merga, Campbell et al. 2014).

1.9.2.3 Tight Junctions

Desomosomes, adherent junctions and tight junctions hold epithelial cells of the gut mucosa together. Tight junctions prevent intercellular ingress by pathogens. They are composed of proteins from four junctional protein families: occluding, claudin, tricellulin and junctional adhesion molecules (Merga, Campbell et al. 2014). In IBD, tight junctions have been shown to be damaged, although these changes may be secondary to the disease, rather than pathogonomic of it (Zeissig, Burgel et al. 2007).

1.9.2.4 Defensins

Defensins, a group of antimicrobial pepetides <100 amino acids in size, are found in most types of epithelial cells. Paneth cells produce large quantities to regulate small-bowel crypt microbiota (Merga, Campbell et al. 2014). In both adult and paediatric CD a reduction in human defensin 5 (HD5) and HD6 has been identified (Wehkamp, Salzman et al. 2005, Perminow, Beisner et al. 2010).

1.9.2.5 Mucosal B Cells

The adaptive immune effector mechanism throughout the gut is an immunoglobulin A (IgA)-producing B-cell system giving rise to secretory IgA antibodies that function at the mucosal surface, inhibiting both microbial colonisation and antigen penetration through the epithelial barrier. The generation of secretory IgA antibodies in the gut depends on mucosal IgA-producing immunocytes, B-cell blasts and plasma cells that accumulate in the mucosal lamina propria by selective homing mechanisms after being primed by GALT (Brandtzaeg, Carlsen et al.). As well as IgA antibodies, IgG, IgM, IgD and IgE antibodies are also produced. In IBD there is an increase in the number of plasma cells in a non-uniform picture; IgA, IgM, and IgG producing plasma cell increase 2-, 5-, and 30-fold respectively (MacDermott, Nash et al. 1981).

1.9.2.6 Mucosal T Cells

Maintenance of the inflammatory response is mainly mediated by abnormally activated effector $CD4^+$ T helper (Th) cells. In IBD, reduced rates of apoptosis of these cells have been reported (Bouma, Strober 2003). As a result there is upregulation of the synthesis and release of proinflammatory mediators including reactive oxygen and nitrogen metabolites, eicosanoids, chemokines and cytokines (Strober, Fuss 2011).

The differentiations in specific T-cell types depends on the interactions of specific cytokines with signal transducer and activator of transcription (STAT) factors. The cytokines that promote Th cell polarisation are derived from innate immune cells that recognise microbe-associated molecular patterns (MAMPs).

Activated Th cells are divided into 2 subsets: Th-1 and Th-2. Th-1 cells secrete IFN-γ, a potent activator of intracellular killing by macrophages, with the main role of protecting the host against intracellular pathogens, some of which are capable of surviving and replicating within macrophages. Th1 cell development requires the sequential actions of STAT1 and STAT4, induced by IFN-γ and IL-12 respectively, which promote an enhanced expression of the T-box transcription factor normally expressed in T cells, T-bet. Th-2 cells express the transcription factor GATA binding protein 3 (GATA-3) and secrete IL-4, IL-5 and IL-13, collaborating to provide host defence against helminths (Galvez 2014).

CD has traditionally been linked with Th1 cells, with a predominance of IL-12 and IFN-γ in the mucosa, whereas UC is linked to Th2 cells with an increased production of IL-5 and IL-13 (Pallone, Monteleone 1998).

More recently Th17 cells have been described as a new subset of effector Th cells (Langrish, Chen et al. 2005). They are characterised by the expression of transcription factor retinoic acid orphan receptor (ROR)γt, but not T-bet or GATA-3, and by their ability to selectively produce high levels of IL-17A and IL-17F (Ivanov, McKenzie et al. 2006).

1.9.2.7 Macrophages

Macrophages are essential for intestinal homeostasis. The macrophage pool in the human intestine is heterogeneous, with its exact nature being determined by the presence or absence of inflammation. Despite being actively phagocytic and bacteriacidal, resident mucosal macrophages do not produce pro-inflammatory mediators in response to stimuli such as TLR ligands. Instead they act as non-inflammatory scavengers of bacteria, as well as assisting in the maintenance of regulatory T cells and promoting epithelial cell renewal, via the production of IL-10 and PGE_2 (Prostaglandin E2) respectively (Bain, Scott et al. 2013).

In IBD, macrophages produce inflammatory mediators such as TNFα, IL-1, IL-6 and nitric oxide (MacDonald, Monteleone et al. 2011). It is thought that distinct populations of intestinal macrophages are responsible for their functional plasticity under different condictions: “resident” and “inflammatory” lineages of monocytes (Geissmann, Manz et al. 2010). In IBD, the number of macrophages of the colon is increased, partly from proliferation of the resident macrophage population, but more so from the recruitment of new monocytes / macrophages from peripheral blood (Rugtveit, Brandtzaeg et al. 1994).

1.9.2.8 Epithelial Cells

The oral cavity, oesophagus and anal canal are lined with stratified squamous epithelium. The remaining gut is lined with nonciliated coloumnar epithelium, which is involved in the absorption of water and nutrients, as well as acting as a barrier to luminal pathogens.

The epitheilial monolayer is folded, producing crypts and villous protrusions. The crypts are the site of intestinal epithelial cell differentiation into Paneth cells, remaining at the base of the crypt, and

goblet, enteroendocrine, and absorptive cells that migrate to the tip of the villous. The epithelium is intermittently interrupted by Peyer's patches, lymphoid aggregates, which are overlaid by microfold (M) cells, adept at sampling and transcytosing luminal antigen (Henderson, van Limbergen et al. 2011).

Enterocytes are hyperpolarised columnar epithelial cells. Not only do they produce a physical barrier to antigens, but also can act independently as antigen-presenting cells (APCs). In order to do this efficiently, intestinal epithelial cells express major histocompatibility complex (MHC) molecules class II (Bland 1988) and also class I molecules (Shao, Kamalu et al. 2005).

The epithelium produces antimicrobial proteins such as lysozymes, secretory phospholipid A_2, defensins, cathelicidins, RNases and C-type lectins in order to prevent microbial invasion (Henderson, van Limbergen et al. 2011). Cathelicidin LL-37 has been shown to be upregulated in UC (Schauber, Rieger et al. 2006) and C-type lectin MGL1/CD301has an anti-inflammatory role in murine explerimental colitis through the upregulation of IL-10 (Saba, Denda-Nagai et al. 2009). A SNP (Single Nucleotide Polymorphism) in the region of *CLEC16A* that encodes a protein containing C-type lectin domain, has been shown to be associated with CD patients lacking the NOD2/CARD15 mutations (Marquez, Varade et al. 2009).

1.9.2.9 Endothelial Cells

Endothelial cells (ECs) are the major constituent of the microvasculature that line blood vessels and lymphatics.

In health, ECs provide an anti-adhesive, selectively permeable exchange barrier (Cines, Pollak et al. 1998). However, in IBD ECs undergo rapid changes in response to elevated levels of cytokines and growth factors related to gut injury. The endothelial microvasculature both expands and increases in permeability (Oshima, Laroux et al. 2001).

ECs can produce IL-1β, IL-3 and IL-6 upon stimulation with inflammatory cytokines, TNFα, and IL-1 (Nilsen, Johansen et al. 1998). ECs show distinct properties such as constitutive inducible nitric oxide synthase (iNOS) and unique adhesive determinants that may be altered in IBD (Binion, Rafiee et al. 2000). Endothelial-derived NO reduces leukocyte and platelet adhesion to the endothelium (Sessa 2009, Binion, Fu et al. 1998), mediates flow-dependent and agonist-dependent vasodilatation, and couples VEGF-A (vascular epithelial growth factor A) signalling with NO-dependent permeability (Petersson, Schreiber et al. 2007, Spyridopoulos, Luedemann et al. 2002).

Endothelial nitric oxide synthase (eNOS)-derived NO is a radical scavenger that absorbs O_2 and generates ONOO (perioxynitrite), a potent oxidant (Forstermann, Munzel 2006). eNOS expression is reduced in IBD, reducing endothelium-dependent vasodilatation, leading to ungoverned oxidant formation (Hatoum, Binion et al. 2003). NO may prevent development of endothelial inflammatory and hyper-adhesive phenotype in IBD by suppressing cytokine-induced EC adhesion molecules (ECAMs) and matrix mettaloproteinases (MMPs) (Oshima, Jordan et al. 2001). Increases in endothelial oxidant stress disturbs tight junctional organisation via p38, p42/44 MAPK (mitogen-activated protein kinase) (Kevil, Oshima et al. 2000).

ECs from CD patients show a persistent loss of iNOS expression (Binion, Rafiee et al. 2000). iNOS can be decreased by injury to normal ECs, the opposite of most tissues, which mobilise iNOS in response to injury (Binion, Rafiee et al. 2000). Despite decreased iNOS however, IBD frequently exhibits increased leucocyte recruitment and activation of gut epithelial cells to increase overall NO production (Oshima, Jordan et al. 2001). Tissue-derived iNOS and leucocyte iNOS mediate colitis injury, and iNOS-dervived NO plays an important role in gut healing after injury through induction of VEGF, necessary for angiogenesis in wound healing (Aoi, Terashima et al. 2008). Excess NO may drive gut injury and thus exacerbate IBD (Elrod, Laroux et al. 2005).

Endothelial cells of the gut microvasculature are activated by Toll-like receptor (TLR) signalling. Protease activated receptors activate transforming growth factor (TGF)-β to induce TLR4 and lead to increased IBD severity (Cromer, Mathis et al. 2011). TLR5, a receptor for flagellin, is expressed in all ECs (Maaser, Heidemann et al. 2004). TLR5 signalling induces endothelial intercellular adhesion molecule-1, TNFα production and leukocyte binding and emigration. Loss of TLR5 leads to infectious colitis due to deficent and improper responses to normal flora and pathological microorganisms (Vijay-Kumar, Aitken et al. 2008, Vijay-Kumar, Sanders et al. 2007).

TLR3, mediated by interferon (IFN) type 1 induction of IL-10, a potent anti-inflammatory cytokine, is protective in the murine model of acute colitis (Vijay-Kumar, Wu et al. 2007).

In IBD, plasma levels of inflammatory cytokines such as IL-6, IL-23, IL-12, and TNFα are increased (Cromer, Mathis et al. 2011). TNFα has pleiotropic effects of the endothelium in IBD, ranging from the induction of adhesion molecules (vascular cellular adhesion molecule (VCAM)-1, and mucosal addressin cellular adhesion molecule (MAdCAM)-1), the promotion of interaction of platelets with ECs, and inducing expression of pro-angiogenic growth factors such as VEGF-A (Oshima, Jordan et al. 2001, Haraldsen, Kvale et al. 1996).

Defects in the activity of the anti-inflammatory cytokines such as IL-10 may play a role in establishing IBD. Pretreatment of ECs with IL-10 prevents IFN-γ mediated endothelial barrier disruption (Oshima, Laroux et al. 2001). Several EC adhesion molecules such as intercellular adhesion molecule (ICAM)-1, VCAM-1 (vascular cell adhesion molecule 1) and MAdCAM-1 are increased in IL-$10^{-/-}$ mouse colitis and may mediate leukocyte recruitment in this model (Kawachi, Jennings et al. 2000).

In IBD, increases in both blood and lymphatic vessels in the intestine increases the endothelial surface area, increasing leukocyte recruitment with the mobilisation of ECAMS, including selectins (Binion, West et al. 1998). P and E-selectins, glycoproteins expressed on the surface of platelets and other leukocytes, are also expressed on the surface of activated or inflammed endothelium in IBD. P-selectin, a partial mediator of gut-infiltrating leukocytes, interacts with ECAMs such as VCAM-1 / ICAM-1, as well as O-glycans (Davenpeck, Gauthier et al. 1994, Kobayashi, Fukuda et al. 2009). Increased platelet P-selectin, with the enhanced prothrombotic surface of the gut EC in IBD, increases thrombus formation and tissue damage by ischaemic injury (Fagerstam, Whiss 2006). E-selectin is expressed solely on the surface of activated ECs during inflammation and is a major contributor to

leukocyte rolling injury. It is not stored but instead must be produced in response to inflammatory stimuli such as IL-1, TNFα, and VEGF-A (Rho, Chung et al. 2009, Zittermann, Issekutz 2006).

Activation of the clotting cascade, as well as platelet and leukocyte activation in IBD reflect the loss of the non-thrombogenic EC phenotype. Thrombi aggravate inflammation by binding of micro infarcts to the endothelial surface leading to ischaemic inflammation in the intestinal microvasculature (Tabibian, Roth 2009).

1.9.2.10 Enteroendocrine Cells

Gut enteroendocrine cells (EEC) act as sensors of luminal content via G-protein coupled "taste" receptors (T1R2, T1R3 and T2R) and nutrient (SCFA) receptors (GPR40, GPR41, GPR43 and GPR120). They also express sensory receptors of the mucosal innate immune system. They function as transepithelial signal transduction conduits by releasing regulatory peptides and amines such as cholecystokinin (CCK, glucagon-like peptide-1 and -2 (GLP-1/2), and polypeptide YY. EECs via GLP-2 are involved in epithelial homeostatsis and repair (Moran, Pennock et al. 2012). Two EEC genes have been linked to CD: ubiquitination protein 4a (UbE4A) (Sakiyama, Fujita et al. 2008) and Phox2B (Rioux, Xavier et al. 2007).

1.9.2.11 Development of Future Therapies

Treatment of IBD to inhibit EC functions such as immune cell recruitment and inflammatory angiogenesis, and to improve beneficial lymphatic function may be developed. The use of endogenous inhibitors of leukocyte binding (sVCAM) and peptides (AJM300), as well as of angiogenesis (VEGF164b) may become novel therapies (Cromer, Mathis et al. 2011).

1.9.3 Genetics

In IBD, large quantities of research time and money have been utilised in genetic studies, which have lead to a greater understanding of both disease inheritance and susceptibility, and gut homeostasis.

1.9.3.1 Twins Studies

Twin studies have been carried out in IBD and have provided useful information regarding disease pathogenesis. If a disease has a purely genetic origin, concordance between monozygotic (MZ) twins should approach 100%. In dizygotic (DZ) twins you would expect the concordance to be 50%. Should a disease have a purely environmental origin, you would expect the concordance to be similar in both sets of twins.

Europeans studies have shown concordance in monozygotic twins to be 20-58% for CD, and 14-19% for UC, whereas in dizygotic twins concordance rates of ≤7% have been shown in both CD and UC (Tysk, Lindberg et al. 1988, Thompson, Driscoll et al. 1996, Orholm, Binder et al. 2000, Halfvarson, Bodin et al. 2003, Spehlmann, Begun et al. 2008). Halfvarson has recently re-run the Swedish twin registry data with the Swedish hospital discharge register and found the concordance rate in monozygotic twins for CD to be 27% (Halfvarson 2011), previously thought to be 58% (Tysk,

Lindberg et al. 1988). Rates of CD in dizygotic twins was calculated at 2% (Halfvarson 2011), previously thought to be 6% (Tysk, Lindberg et al. 1988). Concordance for mono- and dizygotic twins with UC was 15% and 6% respectively, not representing a significant change from previous. This study suggests that the influence of genetics in CD may previously have been overestimated.

1.9.3.2 Family Studies

Familial clustering of IBD has been extensively studied. Patients with CD report a family history of CD in a first degree relative in 2 – 14% of cases (Orholm, Munkholm et al. 1991, Peeters, Nevens et al. 1996), and a relative with IBD in 5 – 16% of cases (Orholm, Munkholm et al. 1991, Halme, Turunen et al. 2002). Those with UC report a family history in a first degree relative of UC in 7 – 11% (Halme, Turunen et al. 2002, Probert, Jayanthi et al. 1993, Yang, McElree et al. 1993) and IBD in 8 – 14% of cases (Orholm, Munkholm et al. 1991, Halme, Turunen et al. 2002).

Potentially these figures may be biased, as most published studies come from teaching hospitals that may see a more severe disease phenotype, and therefore have more family members affected than a population based study. The other confounder is that the longer studies go on, the more chance there is of a family member developing IBD.

The use of age-adjusted figures is a method to establish the relative risk of developing IBD. The most commonly used method is Strömgren's. Calculations are based on the assumption that everyone will live to 70 years of age. Based on work from Europe and North American (Orholm, Munkholm et al. 1991, Peeters, Nevens et al. 1996, Yang, McElree et al. 1993), the approximated age-adjusted life-time risk of developing IBD for a non-Jewish first-degree relative of a person with CD is 5 % and 1.6% for UC (Halme, Paavola-Sakki et al. 2006). In Jewish populations these figures are calculated to be 8% and 5.2% respectively (Yang, McElree et al. 1993).

Offspring of two parents with IBD are at increased risk of developing IBD with studies showing that up to 36% may be affected (Bennett, Rubin et al. 1991).

Despite the different methodologies employed, it has been consistently shown that first degree relatives are at greatest risk of developing IBD, especially siblings, while parents are at lowest risk (Peeters, Nevens et al. 1996, Yang, McElree et al. 1993), and that a positive family history is seen more often in CD than in UC (Halme, Paavola-Sakki et al. 2006).

1.9.3.3 Family-linkage Studies

As previously discussed, twin studies demonstrated important genetic components to complex diseases such as IBD, however there was difficulty isolating the specific loci involved. Family-linkage studies, the study of large families with disease throughout multiple generations, are useful in the mapping of Mendelian disease genes, but were not optimal in the investigation of complex diseases such as IBD. With the exception of NOD2 and CD (Hugot, Chamaillard et al. 2001, Ogura, Bonen et al. 2001), it became apparent that these complex diseases are not caused by a few highly penetrant mutations, but were instead affected by a range of both common and rare mutations that only moderately affect disease risk.

1.9.3.4 Association Studies

Association studies, the comparison of a disease population with a healthy population in relation to genetic variations, allowed further progress to be made in the study of IBD, however it was not until genome-wide association studies became commonplace that knowledge of the genome rapidly evolved in IBD studies.

1.9.3.5 Candidate Studies

Candidate genetic studies allow researchers to focus on a single gene that has previously been identified through other study methodologies or prior knowledge. The advantage is that by either finding, or refuting an association, evidence for or against the functionality of the variant in a specific pathway can be clarified. The disadvantages of this method are that prior knowledge, which may be subjective, is required. Large study samples may be essential to identify the function of a single genetic variant in a complex disease, and because the search for variants is limited to a specific region, the influence of causative agents out with this region will be missed.

1.9.3.6 Autophagy

Autophagy is an essential process required for cellular homeostasis and organelle turnover (Rioux, Xavier et al. 2007) and occurs through the removal of waste products from the cellular cytoplasm. This occurs by encapsulation of the products to form an autophagosome, which eventually fuses with lysosomes causing degradation and recycling of the vesicle contents. The antigens are loaded on to major histocompatibility complex class II to activate T cell receptor-mediated adaptive immune responses (Plantinga, Crisan et al. 2011). This lysosomal degradation pathway is also used to degrade microorganisms that invade the cell such as bacteria, viruses and protozoa (Levine, Mizushima et al. 2011).

Autophagy is associated with most cellular stress-response pathways, including those involved in controlling immune responses and inflammation. This complex process also involves interactions between autophagy proteins and immune signalling molecules. An intricate relationship exists between the autophagy pathway and proteins, and immunity and inflammation. Autophagy proteins function simultaneously in the induction and suppression of immune and inflammatory responses, and vice versa, and therefore defects in autophagy genes may lead to inflammatory conditions (Levine, Mizushima et al. 2011).

Genetic variation in genes involving the recognition of bacteria (NOD2), and also autophagy genes (ATG16L1 and IRGM) (Rioux, Xavier et al. 2007) involved in the processing and elimination of bacteria may provide insight into Crohn's disease pathogenesis.

1.9.3.7 NOD2/CARD15

The first genome wide linkage study in CD was reported in 1996 and identified genomic sharing between CD relatives on chromosome 16, IBD1 (Hugot, Laurent-Puig et al. 1996). Since then there

has been mass replication of this finding with NOD (nucleotide oligomerisation domain) 2, also known as CARD (caspase-activation recruitment domain) 15 or IBD1 on chromosome 16 being confirmed as linking with CD but not UC (Hugot, Chamaillard et al. 2001, Ogura, Bonen et al. 2001, Cavanaugh, IBD International Genetics Consortium 2001, Hampe, Cuthbert et al. 2001).

The NOD2 gene was first characterised as belonging to a family of proteins sharing structural homologies to the plant R proteins that mediate host resistance to pathogens (Ogura, Inohara et al. 2001). The NOD2 gene contains two N terminus caspase-activation recruitment domains, a central nucleotide oligomerisation domain and a C terminus leucine- rich repeat (LRR) domain. The LRR domain mediates host response to microbial stimulation for both NOD and toll-like receptor (TLR) proteins (Khor, Gardet et al. 2011). NOD2 is expressed in intestinal epithelial cells, with particularly high expression in Paneth cells in the small intestine, intestinal myofibroblasts, endothelial cells, granulocytes, and monocyte-derived cells, including macrophages, osteoblasts, and dendritic cells (Cho, Abraham 2007). Activation of NOD2 results in activation of multiple signaling pathways, including the NF-κB and MAPK pathways, and ultimately leads to a variety of immune responses. Within NOD2, three coding region variants in or near the LRR domain, Arg702Trp, Gly908Arg, and the frameshift mutant Leu1007fsinsC, are each independently associated with CD. The frameshift mutant truncates the terminal 33 amino acids of the protein, resulting in a marked loss in the capacity to activate NF-κB (Ogura, Bonen et al. 2001).

NOD2, a pattern recognition receptor (PRR), directly binds ATP (adenosine triphosphate) and muramyl dipeptide (MDP), a breakdown product of peptidoglycan derived from the cell wall of Gram-negative and Gram-positive bacteria. NOD2 recognises MDP, which modulates both innate and adaptive immune responses (Shaw, Kamada et al. 2011). MDP stimulation induces autophagy, which controls bacterial replication and antigen presentation, and acts on dendritic cells in conjunction with TLR ligands to promote TH17-cell differentiation (Cooney, Baker et al. 2010, Travassos, Carneiro et al. 2010). NOD2 may also contribute to immune tolerance. These effects are impaired in cells from patients with the Crohn's-disease-associated NOD2 mutation 3020insC. Furthermore, NOD2 can participate in distinct MDP-independent pathways such as regulation of the T-cell response and the type I IFN (interferon) response to single-stranded RNA (ssRNA) stimulation, indicating that gut microbial ssRNAs may exist and have immunomodulatory properties (Sabbah, Chang et al. 2009).

Previously it was thought that carriers of either homozygous or compound heterozygous NOD2/CARD15 variant genotypes are associated with a severe, early onset disease phenotype consisting of stricturing and/or fistulating disease (Lesage, Zouali et al. 2002, Laghi, Costa et al. 2005), the requirement for early surgical intervention, and surgical recurrence (Alvarez-Lobos, Arostegui et al. 2005). However, the most recent, and largest, genotype-phenotype IBD study encompassing phenotyping from nearly 30 000 IBD patients worldwide and matching genotypes from 150 000 varients, has shown that in CD, NOD2 is not associated with stricturing disease after accounting for disease location (Cleynen, Boucher et al. 2016). Significant associations between ileal CD and NOD2 were identified.

1.9.3.8 ATG16L1

Autophagy-related 16-like 1 gene (ATG16L1) encodes for one of the main proteins involved in the formation of autophagosomes (Plantinga, Crisan et al. 2011). A mutation in ATG16L1 (non-synonymous polymorphism T300A) has been implicated in susceptibility to Crohn's disease (Rioux, Xavier et al. 2007, Hampe, Franke et al. 2007). As with NOD2 variants, dendritic cells from patients with the Crohn's-disease-associated *ATG16L1*(*T300A*) risk variant are defective in presenting bacterial antigen to $CD4^+$ T cells. In addition, ATG16L1 may have unique protective functions, including Paneth cell antimicrobial peptide release and the negative regulation of pro-inflammatory cytokine production (Plantinga, Crisan et al. 2011).

1.9.3.9 IRGM

The IRGM (immunity-related GTPase family M) gene belongs to immunity-related GTPases, a family of genes induced by interferons and functioning as key mediators of IFN-regulated resistance to intracellular bacteria and protozoa (Singh, Davis et al. 2006). Genome-wide Association Studies (GWAS) have shown IRGM to be strongly associated with CD (Parkes, Barrett et al. 2007, Wellcome Trust Case Control Consortium 2007). In CD patients, autophagy has been demonstrated to limit the replication of intracellular adherent-invasive *Escherichia coli* (AIEC) associated with ileal CD. IRGM and ATG16L1-deficient cells have enhanced intracellular AIEC bacteria replication, suggesting a significant impact on the outcome of intestinal inflammation (Lapaquette, Glasser et al. 2010).

1.9.3.10 HLA

The major histocompatibility complex (MHC), known in humans as the human leukocyte antigen (HLA) region, was first reported on with regards to IBD in 1972 (Gleeson, Walker et al. 1972). Since then numerous studies have investigated the role HLA gene may play in IBD. HLA was one of the first multi-megabases regions to be sequenced (Complete sequence and gene map of a human major histocompatibility complex. The MHC sequencing consortium. 1999). HLA spans approximately 4 megabases of DNA in the chromosome band 6p21.3. It is divided into 3 classes, and contains 224 densely packed highly polymorphic gene loci, mainly relating to immunologic functions (Yap, Ahmad et al. 2004). Polymorphisms in key areas such as peptide binding regions and T cell receptor repertoire determination may affect these functions, possibly causing disease. A possible functional polymorphism is that of the association of the HLA DRB1*0103 allele with IBD. This allele encodes an HLA DR molecule with a unique sequence within the peptide-binding groove, thus allowing it to bind and present potentially disease-associated peptides (Coppin, Carmichael et al. 1993).

HLA Class I and II genes have been extensively investigated through linkage studies to determine associations with IBD, however, results have been inconsistent. In UC evidence was found for linkage to HLA DRB1 (Satsangi, Welsh et al. 1996).

Since then advances in technology and GWAS has allowed for the further investigation of HLA genes with regards to IBD. The focus has been on HLA Class II genes as they play a pivotal role in immune function. The class II molecules consist of an α chain and a β chain that form a groove in which the

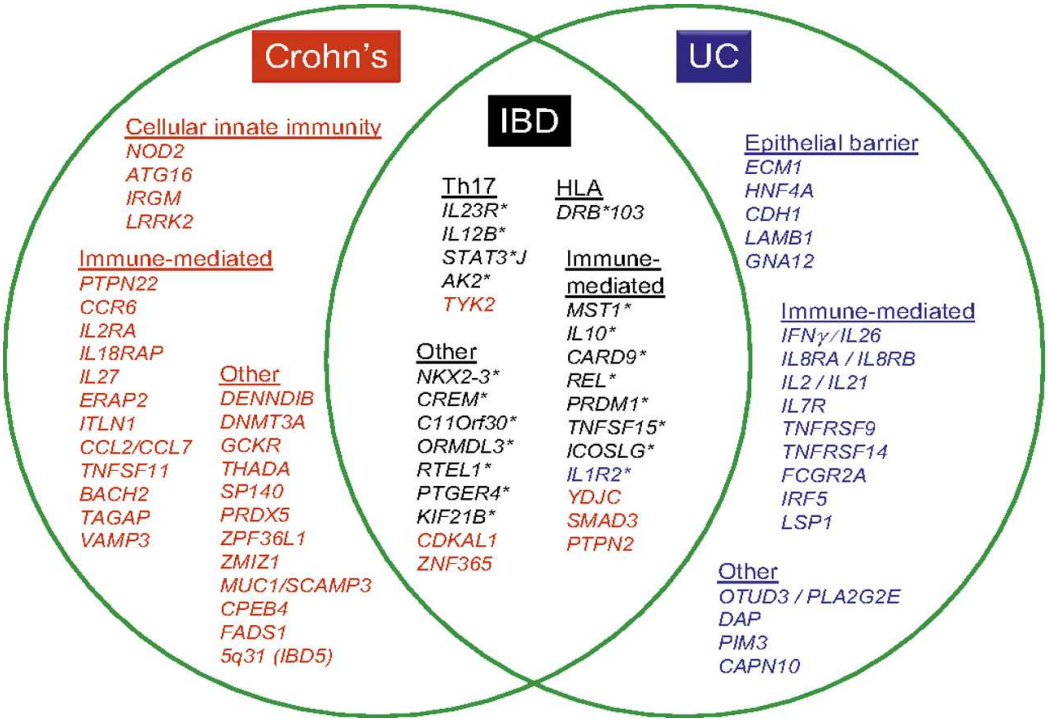
antigenic peptide, after partial digestion of antigen by antigen presenting cells, is conferred to the T cell receptor (Brown, Jardetzky et al. 1993). Meta-analysis has shown a positive association of HLA-DR9, HLA-DR2 and allele 1502 of its split antigen HLA-DR15 with UC but a protective effect of HLA-DR4 against UC. HLA-DR7, HLA-DRB3*0301 and DQ4 (when Japanese populations included) are positively associated with CD, with HLA-DR2 and HLA-DR3 negatively associated with CD (Stokkers, Reitsma et al. 1999).The HLA-DRB1*0103 allele has been positively associated with UC, and has been shown to predispose to extensive disease (Roussomoustakaki, Satsangi et al. 1997). Studies in CD have shown associations with DR1 (Toyoda, Wang et al. 1993, Danze, Colombel et al. 1996), DR4 (Matake, Okabe et al. 1992), and DR7 (Danze, Colombel et al. 1996, Reinshagen, Loeliger et al. 1996) with a protective effect of DR3 (Danze, Colombel et al. 1996, Reinshagen, Loeliger et al. 1996). The HLA-DRB1*0103 allele is also associated with CD and predisposes to later age at diagnosis and colonic disease compared to HLA-DRB1*0701 and HLA-DRB1*04 predicting ileal disease (Newman, Silverberg et al. 2004).

Most recently, the association between MHC and colonic CD has been confirmed, as has the association between MHC and both extensive and severe disease requiring colectomy in UC (Cleynen, Boucher et al. 2016).

1.9.3.11 GWAS

Conversely, genome-wide association studies are essentially a hypothesis-free methodology utilising high-throughput technology to genotype single nucleotide polymorphisms (SNPs) in large meticulously phenotyped patient cohorts, prompting the rapid discovery of complex disease genetics. Both CD and UC have been extensively investigated through international collaborative groups (International Inflammatory Bowel Disease Genetics Consortium, http://www.ibdgenetics.org/) amassing large datasets and thus huge statistical power (de Bakker, Ferreira et al. 2008). Most recently the number of confirmed IBD loci is 163, with each loci containing an average of 5 genes (Jostins, Ripke et al. 2012), the most in any complex disease. 110 of the 163 loci are associated with both CD and UC phenotypes, 30 are specific to CD and 23 to UC respectively. 43 of these 53 loci show the same direction of effect in the non-associated disease. Risk alleles at two CD loci, PTPN22 and NOD2, show significant protective effects in UC, potentially reflecting biological differences between the two diseases. In fact, the same coding variant of PTPN22 is a strong risk factor for type I diabetes and rheumatoid arthritis but is protective against CD (Wang, Baldassano et al. 2010) illustrating one of the many crossovers between genetic aspects of immunological diseases.

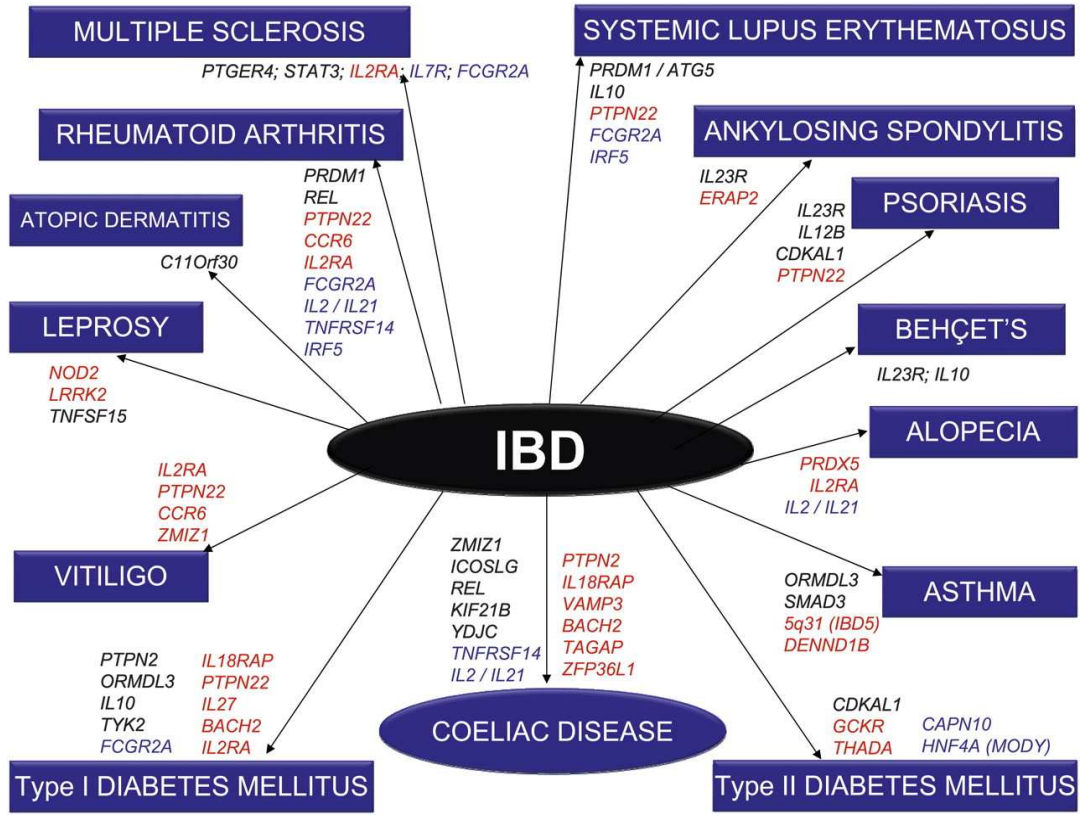
Figure 1.4: IBD Susceptibility Loci (Lees, Barrett et al. 2011)



The loci (depicted by lead gene name) attaining genome-wide significance ($p<5x10^{-8}$) are shown for CD (red), UC (blue) and IBD (black), where $p<5x10^{-8}$ in CD and UC; red where $p<5x10^{-8}$ in CD and $p< 5x10^{-4}$ in UC; blue where $p<5x10^{-8}$ in UC and $p<5x10^{-4}$ in CD.

In the most recent GWAS meta-analysis data, 113 of the 163 IBD loci identified are shared with other diseases or traits. Comparing the overlap with other diseases however is problematic as it depends on the power of other studies. Type I diabetes shares the largest number of loci with IBD (20/39), however this is in part due to the large number of associations known in type I diabetes. Psoriasis (14/17) and ankylosing spondylitis (8/11) also show strong overlaps. IBD loci were shown to be enriched in genes involved with primary immunodeficiencies (PIDs), characterised by immune dysfunction resulting in severe infections. The genes involved in this overlap correlate with reduced circulating T-cell levels (*ADA, CD40, TAP1/2, NBS1, BLM, DNMT3B*), or with specific subsets such as Th17 (*STAT3*), memory (*SP110*), or regulatory T-cells (*STAT5B*). A group of PIDs genes leading to Mendelian susceptibility to mycobacterial disease (MSMD) was also shown to be enriched with six of the eight known autosomal genes linked to MSMD located within IBD loci (*IL12B, IFNGR2, STAT1, IRF8, TYK2* and *STAT3)* and a seventh (*IFNGR1)* almost showing significance. Unsurprisingly perhaps, overlap with IBD is seen in complex mycobacterial disease and IBD associations were found in 7/8 loci identified in leprosy (Jostins, Ripke et al. 2012).

Figure 1.5: Diseases Showing Genetic Overlap with IBD (Lees, Barrett et al. 2011)



Genes that have attained genome-wide levels of significance in all diseases are depicted. IBD loci (black); CD only loci (red); UC only loci (blue).

Table 1.3: Crohn's Disease Loci (Jostins, Ripke et al. 2012)

Crohn's Disease			
Chromosome	**Position (Megabase)**	**SNP**	**Key genes (+ no. of additional loci genes)**
1	78.62	rs17391694	(5)
1	114.3	**rs6679677** ‖	**PTPN22** ¶ (8)
1	120.45	rs3897478	ADAM30 (5)
1	172.85	**rs9286879**	**FASLG, TNFSF18** (0)
2	27.63	**rs1728918**	UCN (23)
2	62.55	rs10865331	(3)
2	231.09	**rs6716753**	SP140 (5)
2	234.15	**rs12994997**	**ATG16L1** ¶ (8)
4	48.36	rs6837335	(6)
4	102.86	rs13126505	(1)
5	55.43	rs10065637	**IL6ST, IL31RA** (1)
5	72.54	rs7702331	(4)
5	173.34	rs17695092	CPEB4 (2)
6	21.42	rs12663356	(3)
6	31.27	**rs9264942**	(22)
6	127.45	rs9491697	(3)
6	128.24	**rs13204742**	(2)
6	159.49	**rs212388**	TAGAP (5)
7	26.88	rs10486483	(2)
7	28.17	rs864745	CREB5, JAZF1 (1)
8	90.87	rs7015630	RIPK2 (4)
8	129.56	**rs6651252**	0
13	44.45	**rs3764147**	LACC1 (3)
15	38.89	rs16967103	**RASGRP1, SPRED1** (2)
16	50.66 †	**rs2066847** ‖	**NOD2** ¶ (6)
17	25.84	**rs2945412**	**LGAS9, NOS2** (3)
19	1.12	**rs2024092**	GPX4, HMHA1 (20)
19	46.85 ‡	rs4802307	(9)
19	49.2	**rs516246**	FUT2, (25)
21	34.77	**rs2284553**	**IFNGR2, IFNAR1** (10)

Table 1.4: Ulcerative Colitis Loci (Jostins, Ripke et al. 2012)

Ulcerative Colitis			
Chromosome	**Position (Megabase)**	**SNP**	**Key genes (+ no. of additional loci genes)**
1	2.5	rs10797432	**TNFRSF14** (10)
1	20.15 †	**rs6426833**	(9)
1	200.09	**rs2816958**	(3)
2	198.65	rs1016883	RFTN2, PLCL1 (7)
2	199.70 *	**rs17229285**	0
3	53.05	rs9847710	PRKCD, ITIH4 (8)
4	103.51	rs3774959	**NFKB1, MANBA** (2)
5	0.59	rs11739663	SLC9A3 (8)
5	134.44	rs254560	(6)
6	32.595	**rs6927022**	(15)
7	2.78	**rs798502**	**CARD11, GNA12** (5)
7	27.22 ‡	rs4722672	(14)
7	107.45 *	**rs4380874**	DLD (9)
7	128.57	**rs4728142**	**IRF5** ¶ (13)
11	96.02	rs483905	JRKL, MAML2 (2)
11	114.38	**rs561722**	NXPE1, NXPE4 (5)
15	41.55	rs28374715	(11)
16	30.47	rs11150589	**ITGAL** (20)
16	68.58	rs1728785	ZFP90 (6)
17	70.64	rs7210086	(3)
19	47.12 ‡	rs1126510	CALM3 (14)
20	33.8	rs6088765	(11)
20	43.06	**rs6017342**	ADA, HNF4A (9)

Table 1.5: General IBD Loci (Jostins, Ripke et al. 2012)

IBD			
Chromosome	**Position (Megabase)**	**SNP**	**Key genes (+ no. of additional loci genes)**
1	1.24	**rs12103**	TNFRSF18, TNFRSF4 (30)
1	8.02	**rs35675666**	TNFRSF9 (6)
1	22.7	**rs12568930** §	(3)
1	67.68 †	**rs11209026** §	**IL23R** ¶ (5)
1	70.99	**rs2651244** §	(3)
1	151.79	**rs4845604** §	RORC (14)
1	155.67	rs670523 §	(31)
1	160.85	rs4656958 §	**CD48** (15)
1	161.47	**rs1801274** §	**FCGR2A, FCGR2B, FCGR3A** (13)
1	197.6	**rs2488389**	C1orf53 (2)
1	200.87	**rs7554511**	KIF21B (6)
1	206.93	**rs3024505** §	IL10 (10)
2	25.12	**rs6545800** §	ADCY3 (6)
2	28.61	**rs925255** §	FOSL2, BRE (1)
2	43.81	rs10495903 §	(5)
2	61.2	**rs7608910**	**REL** (9)
2	65.67	rs6740462	SPRED2 (1)
2	102.86 *	**rs917997** §	IL18RAP, IL1R1 (7)
2	163.1	rs2111485	IFIH1 (5)
2	191.92	rs1517352	**STAT1, STAT4** (2)
2	219.14	rs2382817	(15)
2	241.57 *	**rs3749171** §	**GPR35** (12)
3	18.76	**rs4256159** §	0
3	48.96 †	**rs3197999**	**MST1, PFKB4** (63)
4	74.85	rs2472649 §	(11)
4	123.22	**rs7657746**	**IL2, IL21** (2)
5	10.69	rs2930047	**DAP** (2)
5	40.38 †	**rs11742570** §	PTGER4 (1)
5	96.24	**rs1363907**	ERAP2, ERAP1 (3)
5	130.01	rs4836519 §	(1)
5	131.19 *	**rs2188962** §	IBD5 locus (18)
5	141.51	**rs6863411** §	SPRY4, NDFIP1 (5)
5	150.27	**rs11741861** §	**IRGM** ¶ (10)
5	158.8 †	**rs6871626** §	**IL12B** (3)
5	176.79	rs12654812	**DOK3** (17)
6	14.71	rs17119	0
6	20.77 *	**rs9358372** §	(2)
6	90.96	rs1847472	(1)
6	106.43	**rs6568421** §	(2)
6	111.82	**rs3851228**	**TRAF3IP2** (4)
6	138	**rs6920220** §	TNFAIP3 (1)
6	143.9	rs12199775	PHACTR2 (5)
6	167.37	**rs1819333** §	**CCR6, RPS6KA1** (4)
7	50.245 *	**rs1456896**	ZPBP, IKZF1 (4)
7	98.75	rs9297145	SMURF1 (6)
7	100.34	**rs1734907** §	**EPO** (21)
7	116.89	rs38904 §	(6)
8	126.53	**rs921720** §	TRIB1 (1)
8	130.62	rs1991866	(2)

9	4.98	**rs10759669**	**JAK2** (4)
9	93.92	rs4743820 §	NFIL3 (2)
9	117.60 †	**rs4246905**	TNFSF15 (4)
9	139.32 *	**rs19781499** §	**CARD9** (22)
10	6.08	rs12722515 §	**IL2RA, IL15RA** (6)
10	30.72	rs1042058 §	**MAP3K8** (3)
10	35.3	**rs11010067** §	**CREM** (3)
10	59.99	rs2790216	CISD1, IPMK (2)
10	64.51 †	**rs10761659** §	(3)
10	75.67	rs2227564 §	(13)
10	81.03	**rs1250546** §	(5)
10	82.25	**rs6586030** §	TSPAN14, C10orf58 (4)
10	94.43	rs7911264	(4)
10	101.28	**rs4409764**	NKX2-3 (6)
11	1.87	rs907611	**TNNI2, LSP1** (17)
11	58.33	rs10896794	CNTF, LPXN (8)
11	60.77	**rs11230563**	**CD6** (14)
11	61.56	**rs4246215** §	(15)
11	64.12	rs559928	**CCDC88B** (23)
11	65.65	rs2231884 §	RELA (25)
11	76.29	**rs2155219** §	(5)
11	87.12	rs6592362	(1)
11	118.74	rs630923 §	CXCR5 (17)
12	12.65	rs11612508 §	LOH12CR1 (8)
12	40.77 *	**rs11564258** §	MUC19 (1)
12	48.2	rs11168249 §	VDR (8)
12	68.49	**rs7134599** §	**IFNG** (3)
13	27.52	**rs17085007** §	(2)
13	40.86 †	**rs941823** §	(3)
13	99.95	**rs9557195**	GPR183, GPR18 (6)
14	69.27	rs194749 §	ZFP36L1 (4)
14	75.7	rs4899554 §	**FOS, MLH3** (6)
14	88.47	**rs8005161**	**GPR65, GALC** (1)
15	67.27	**rs17293632** §	SMAD3 (2)
15	91.17	rs7495132	CRTC3 (3)
16	11.54 *	**rs529866** §	**SOCS1, LITAF** (11)
16	23.86	rs7404095	**PRKCB** (5)
16	28.6	**rs26528** §	IL27 (14)
16	86	rs10521318 §	IRF8 (4)
17	32.59	**rs3091316** §	**CCL13, CCL2** (5)
17	37.91	**rs12946510**	ORMDL3 (16)
17	40.53	**rs12942547** §	**STAT3** (15)
17	57.96	**rs1292053** §	TUBD1, RPS6KB1 (9)
18	12.8	**rs1893217** §	(6)
18	46.39	rs7240004 §	SMAD9 (2)
18	67.53	rs727088	**CD226** (2)
19	10.49 *	**rs11879191**	**TYK2** (27)
19	33.73	**rs17694108**	CEBPG (8)
19	55.38	rs11672983	(19)
20	30.75	rs6142618 §	HCK (10)
20	31.37	rs4911259	DNMT3B (8)
20	44.74	**rs1569723** §	**CD40** (13)
20	48.95	rs913678	CEBPB (5)
20	57.82	rs259964	ZNF831, CTSZ (5)

20	62.34	**rs6062504**	TNFRSF6B (26)
21	16.81	**rs2823286** §	0
21	40.46	**rs2836878** §	(3)
21	45.62	**rs7282490**	ICOSLG (9)
22	21.92	**rs2266959**	(13)
22	30.43	**rs2412970**	**LIF, OSM** (9)
22	39.69 *	**rs2413583** §	TAB1 (18)

In relation to tables 1.3-5, the position given is the middle of the locus window, with all positions relative to human reference genome GRCh37. Bolded rs numbers indicate SNPs with p values less than $1x10^{-13}$. Grey shading indicates newly discovered loci. Listed are genes implicated by one or more candidate gene approaches. Bolded genes have been implicated by two or more candidate gene approaches. For each locus, the top two candidate genes are listed.

* Additional genome-wide significant associated SNP in the region.

† Two or more additional genome-wide significant associated SNPs in the region.

‡ These regions have overlapping but distinct UC and CD signals.

§ Hetrogeneity of odds ratios.

|| CD risk allele is significantly protective in UC.

¶ Gene for which functional studies of associated alleles have been reported.

1.9.3.12 Epigenetics

In humans, monozygotic (MZ) twins account for 1:250 live births (Fraga, Ballestar et al. 2005). Studies in MZ twins, considered genetically identical, have been used in the demonstration of the influence of environmental factors in complex diseases and human phenotypes (MacGregor, Snieder et al. 2000). However, significant discordance can occur in MZ twins for disease types and traits, and the reason potentially could be explained by epigenetic differences. Epigenetic profiles may in fact represent the link between an environmental factor and phenotypic differences in MZ twins.

A popular definition of epigenetics is “the study of mitotically and/or meiotically heritable changes in gene expression that occur without a change in DNA sequence” (Russo V.E.A., Martienssen R.A. et al. 1996). The epigenome of a cell is controlled by complex interactions between genetic and environmental factors (Rakyan, Down et al. 2011). In mammals, epigenetic information can be transferred in mitotically stable DNAm (DNA methylation), post-translational histone proteins and non-coding RNAs (ncRNAs) (Bernstein, Meissner et al. 2007).

1.9.3.12.1 DNA Methylation

DNA methylation is a mechanism for silencing gene expression and maintaining stability during massive DNA replication, which if left unregulated, could allow illegitimate recombination events and cause transcriptional deregulation of nearby genes (Robertson 2005). For DNAm, the most common form of methylation is of cytosines in the context of cytosine-guanine dinucleotides (CpGs). CpG dinucleotides tend to cluster in areas called CpG islands, areas with more than 200 bases with a G+C

content of >50%. Approximately 60% of human gene promoters are associated with CpG islands, which tend to be unmethylated in normal cells (Straussman, Nejman et al. 2009). CpH (where H = C/A/T) methylation also occurs but is less common, as is 5-hydroxymethylation of cytosines catalysed by ten-eleven translocation methycytosine dioxygenases (Rakyan, Down et al. 2011).

1.9.3.12.2 Histone Proteins

Chromatin is a combination of DNA and proteins that form the nucleus of a cell. The nucleosome, the fundamental unit of chromatin, is composed of an octamer of the four core histones (H3, H4, H2A, H2B) around which 147 base pairs of DNA are wrapped. The core histones are predominantly globular except for their N-terminal ‘‘tails’’, which are unstructured and subject to modifications in the form of acetylation, methylation (lysines / arginines), phosphorylation, ubiquitylation, sumoylation, ADP (adenosine diphosphate) ribosylation, deimination or proline isomerisation to one or more of their amino-acids (Kouzarides 2007). Histone modifications play important roles in transcriptional regulation, DNA repair and replication (Huertas, Sendra et al. 2009), alternative splicing (Luco, Pan et al. 2010) and chromosome condensation (Kouzarides 2007). Histone modification levels are predictive for gene expression (Karlic, Chung et al. 2010).

1.9.3.12.3 Nucleosome Positioning

As discussed earlier, the nucleosome is a method of packaging a helical DNA polymer. Nucleosomes are positioned as a linear array along a DNA polymer. The exact position of the nucleosomes in relation to the transcription start sites (TSSs) is very influential on the initiation of transcription (Jiang, Pugh 2009, Portela, Esteller 2010). Small alterations in nucleosome position of only 30 base pairs at TSSs may change the activity of RNA polymerase II. Loss of a nucleosome directly upstream of the TSS is associated with gene activation, and occlusion of the TSS by a nucleosome is associated with gene repression (Schones, Cui et al. 2008, Cairns 2009).

1.9.3.12.4 ncRNA

ncRNAs have been implicated in a variety of epigenetic mechanisms; small RNAs can direct cytosine methylation and histone modifications, microRNAs in gene expression control, large RNAs in X-chromosome inactivation and imprinting, and piRNAs have been implicated in germ cell maintenance (Fabrício F. 2008).

1.9.3.12.5 Epigenetics and IBD

Epigenetic programming starts at fertilisation and continues throughout the lifespan of a being. Epigenetic information is stored as chemical modifications to cytosine bases and to the histone proteins that package the genome. These chemical alterations that regulate chromatin structure and DNA accessibility in turn influence how the genome is made and potentially how the genome interacts with the environment (Bernstein, Meissner et al. 2007). Animal models have shown the effects of epigenetic alterations due to environmental factors in pregnancy to be maintained for up to

two generations (Morgan, Sutherland et al. 1999, Schaible, Harris et al. 2011). This has raised interest in the scientific community where it was previously thought that epigenetic marks were reset during meiosis and therefore the crossing of generations is a novel phenomenon (Faulk, Dolinoy 2011). Factors such as smoking, diet and the microbiota that produce epimutations may now be implicated in the familial predisposition to certain diseases such as IBD (Ventham, Kennedy et al. 2013).

1.10 Biomarkers

Biomarkers are measurable substances in bodily fluids that can be used to diagnose pathology, monitor disease activity, predict disease phenotype, for individual therapeutic targeting, to measure outcomes in clinical trials, or a combination of all of the above (Sargent, Conley et al. 2005). IBD biomarkers include serological, faecal and gene polymorphisms. Their application in the diagnosis and monitoring of IBD is potentially cheaper, less laborious, less invasive and more objective compared to an endoscopy / biopsy based approach (Vermeire, Van Assche et al. 2006), however, currently none of the commercially available biomarkers can be used as stand-alone tools and are simply used as an adjunct to endoscopy. There is a great need for new biomarkers to be developed.

1.10.1 Discovery and Development Challenges

The discovery of biomarkers suitable for clinical use is challenging. Traditionally biomarkers were developed through a hypothesis driven understanding of disease pathophysiology. However, now the introduction of high throughput "omics" technologies not based on *a priori* hypotheses, whilst potentially providing new causal or mechanistic disease information, has also lead to the potential development of biomarkers ahead of the understanding of the biological basis of disease. Concerns have been raised regarding how to validate such markers. The two main areas of validation are technical (or analytical) validation, regarding intrinsic measurement error and analytical sensitivity, and clinical (or field) validation relating to how a certain marker behaves in the population, depending on biological variability within the population (Vineis, Perera 2007).

The areas of three major concerns are:

1. Overfitting – the tendency of models trained on large numbers of variables measured on small numbers of samples to produce extraordinarily high sensitivity and specificity and then fail on independent validation sets
2. Bias – are results due to differences between the disease and the control samples that do not exist in the disease and control populations?
3. Robustness – can results be generalised to appropriate clinical populations? (Brenner, Normolle 2007)

The Early Detection Research Network (Pepe, Etzioni et al. 2001) has laid out five phases of validation to address "robustness":

Phase I. Preclinical / Exploratory – promising directions identified.

Phase II. Clinical assay and validation – clinical assay detects established disease.

Phase III. Retrospective / Longitudinal – biomarker detects disease early before it becomes clinical and a "screen positive" rule is defined.

Phase IV. Prospective / Screening – extent and characteristics of disease detected by the test and the false referral rate are identified.

Phase V. Disease Control – Impact of screening on reducing the burden of disease on the population is quantified.

The importance of robust studies and techniques to develop simple, sensitive, specific, inexpensive and reproducible tests for biomarkers that have a significant clinical utility cannot be underestimated (Newton, Newman et al. 2012). Further guidelines have been proposed to ensure accuracy and clinical applicability by utilising the prospective-specimen-collection, retrospective-blinded-evaluation (PRoBE) design (Pepe, Feng et al. 2008).

In the UK, The Cancer Research UK Biomarker Discovery and Development Committee have defined the following groups of biomarkers, equally applicable to non-cancer processes (Newton, Newman et al. 2012):

Risk assessment / predisposition biomarkers can be used to define individuals or populations at increased risk of developing a disease. The biomarker sample should be as non-invasive as possible, and the test must be sensitive, specific, reproducible, and able to diagnose the predisposition to a disease prior to the pathology developing. These biomarkers may be markers of a genetic predisposition or a measure of exposure to a substance that increases that individual's risk.

Screening / early detection biomarkers are used to aid early diagnosis of a condition, thus facilitating detection at a more treatable stage. Collection of these biomarkers must be acceptable to the screening population, and highly sensitive to avoid unnecessary anxiety and investigation with false positive results.

Diagnostic biomarkers are used to define the type of cancer or disease present in the patient. These tend to be used alongside standard imaging techniques to further facilitate appropriate treatment. Prostate specific antigen, carcinoembryonic antigen and cancer antigen 125 are examples that can also be used to monitor disease and detect recurrence or relapse.

Pharmacological biomarkers are used to assess pharmokinetics, measure the effects of drug treatment on a specific target (e.g. enzyme inhibition, receptor blockade, induction of apoptosis or reduction in angiogenesis), or to demonstrate clinical effects of that drug. These biomarkers can help to facilitate personalised treatment through dose optimisation and reduction of side effects. In Crohn's disease, the thiopurine methyl- transferase (TMPT) gene has a role in identifying the 1:300 patients who are at risk of developing severe myelosuppression when treated with standard thiopurine dosages (Lennard 2002).

Predictive biomarkers can predict a subpopulation of patients who are likely to respond to a given therapy thus allowing more personalised targeting of treatment. Oestrogen and progesterone receptor status in breast cancer, and KRAS (Kirsten Rat Sarcoma Viral Oncogene Homolog) and response to anti-epidermal growth factor receptor therapy in metastatic colorectal cancer are examples in current oncological practice.

Prognostic biomarkers are used to predict the likely course of a disease. This may be the metastatic potential of a cancer and can be used to guide treatment and surveillance decisions, as well as survival prognosis.

In IBD, the "omics" technologies and advances in genetic analyses have lead to the development of a range of putative biomarkers. However, to date, few have proven their clinical utility and validity in the large prospective randomised trials required. In IBD, challenges remain in diagnosis and differentiation between CD and UC, as well as in the monitoring of disease activity and individual response to treatment. Advances in this field could lead to earlier and more accurate diagnosis, as well as personalisation of treatment.

1.10.2 Generic Serum Biomarkers

1.10.2.1 C-Reactive Protein

C-Reactive Protein (CRP) was first discovered in 1930 by Tillett and Francis (Tillett, Francis 1930), and is one of the most important acute phase proteins in humans (Vermeire, Van Assche et al. 2004). An acute phase reaction can be induced by stimuli of various origins: infectious (bacterial, fungal, mycobacterial, or severe viral), inflammatory, stress, tissue necrosis, trauma, childbirth, and neoplasia (Vermeire, Van Assche et al. 2004).

CRP is a pentameric protein, the majority of which is produced by hepatocytes in response to IL-6, IL1-β and TNF-α originating at the site of inflammation or pathology. It consists of 5 identical subunits called protomers. Each protomer contains 2 binding sites for calcium. These allow CRP to bind to its main ligand, phosphocholine. After binding, the CRP-ligand complex will activate the complement cascade (C1 – C9) via C1q. This results in opsonisation and phagocytosis (Vermeire, Van Assche et al. 2004).

Other minor sites of production have been described; peripheral lymphocytes (Kuta, Baum 1986), neurons of patients with Alzheimer's disease (Yasojima, Schwab et al. 2000) and in the thickened intima of atherosclerotic plaques (Yasojima, Schwab et al. 2001).

When considering CRP as a potential biomarker in the diagnosis of IBD it must be taken into consideration that there is marked heterogeneity in CRP response among inflammatory diseases. CD and rheumatoid arthritis are associated with a strong CRP response, whereas others such as systemic lupus erythematosus, dermatomyositis, Sjögren's syndrome, or UC have only a modest to absent CRP response, despite active inflammation (Vermeire, Van Assche et al. 2004).

Shine et al (Shine, Berghouse et al. 1985) assessed gastrointestinal clinic patients, undergoing history, examination and rectal biopsy as well as routine blood tests including CRP and erythrocyte sedimentation rate (ESR). All patients diagnosed with CD had raised CRP and ESR measurements. Of those diagnosed with UC, 50% had raised CRP and ESR levels, and all were diagnosed by rectal biopsy. Of the patients eventually diagnosed with a functional bowel disorder none had raised CRP or ESR levels. It was concluded that the combination of rectal biopsy and measurement of CRP and ESR successfully differentiates between IBD and functional bowel syndrome.

Poullis et al (Poullis, Zar et al. 2002) used a new highly sensitive ELISA to measure CRP levels in patients attending a gastroenterology clinic. They found a cut off value of 2.3mg/l had a sensitivity of 100% and a specificity of 67% in differentiating functional bowel disorders from new cases of IBD.

CRP has also been evaluated as a marker of disease activity. The half-life of CRP is 19 hours, and this is independent of any physiological or pathophysiological factors or of the concentration of CRP in the serum. Thus, the synthesis rate of CRP by hepatocytes is the only factor determining the plasma CRP concentration. Consequently, only liver failure or therapies affecting the acute phase stimulus may decrease CRP (Vermeire, Van Assche et al. 2004).

Fagan et al (Fagan, Dyck et al. 1982) measured CRP and ESR in patients with CD and UC. CRP levels were found to be significantly higher in CD then in UC patients for all categories of disease; mild, moderate and severe. They also found that in CD, CRP levels corresponded closely with clinical and pathological indices of remission, relapse and response to therapy.

Solem et al (Solem, Loftus et al. 2005) found that in CD patients, moderate to severe clinical activity, active disease at colonoscopy and histologically severe inflammation were all significantly associated with elevated CRP levels. In UC/IC patients, CRP elevation was significantly associated with severe clinical activity, increased ESR, anemia, hypoalbuminemia, and active disease at ileocolonoscopy, but not with histological inflammation.

CRP has been used as a predictor of outcomes in IBD. Travis et al (Travis, Farrant et al. 1996) studied UC with severe colitis, all treated with intravenous and rectal hydrocortisone and 27% with cyclosporine. 41% had a complete response by day 7, 29% had a partial response by day 7, and 29% required a colectomy. During the first 5 days stool frequency and CRP level significantly distinguished between outcomes ($p = 0.00625$). On day 3, 85% of patients with a stool frequency > 8 / day, or a stool frequency of 3 – 8 / day and a CRP of > 45mg/l required a colectomy.

CRP has been evaluated as a marker of treatment response. Louis et al (Louis, Vermeire et al. 2002) showed that in patients receiving infliximab therapy, increased CRP at entry was significantly associated with response ($p<0.004$). 46% of patients without a raised CRP do however respond.

In the ENACT-1 trial (Sandborn WJ. Colombel JF. Enns R. Feagan BG. Hanauer SB. Lawrance IC. Panaccione R. Sanders M. Schreiber S. Targan S. van Deventer S. Goldblum R. Despain D. Hogge GS. Rutgeerts P. International Efficacy of Natalizumab as Active Crohn's Therapy (ENACT-1) Trial Group. Evaluation of Natalizumab as Continuous Therapy (ENACT-2) Trial Group 2005) adults with moderate to severe CD were randomly assigned in a 4:1 ratio to receive an intravenous infusion of either 300 mg of natalizumab or placebo at weeks 0, 4, and 8 and were then followed through week 12. This trial did not reach its endpoint due to a large placebo response in comparison with the natalizumab group (49% v 59%). However, the patients with an elevated CRP on entry showed significant benefit of natalizumab over placebo at both weeks 10 and 12.

These trials raise the question as to whether patients with a normal CRP should be offered biological therapy, as those with a raised CRP appear more likely to respond. However, this inevitably would mean that a number of patients without a raised CRP would be denied a treatment that they may respond to, and is not current practice (Vermeire, Van Assche et al. 2004).

1.10.2.2 Erythrocyte Sedimentation Rate

ESR is the rate at which erythrocytes migrate through the plasma. It is influenced by erythrocytes morphology and the plasma constituents such as immunoglobulins (Gabay, Kushner 1999). The concentration of many serum proteins varies, and some have long half-lives, therefore the ESR does not respond rapidly to alterations in clinical status. In UC, correlation between ESR and disease activity is good (Fagan, Dyck et al. 1982, Gabay, Kushner 1999, Dearing, McGuckin et al. 1969, Sachar, Smith et al. 1986, Sachar, Luppescu et al. 1990), however the ESR may be normal in proctitis and proctosigmoiditis (Desai, Faubion et al. 2007). In CD the ESR correlates less well with disease activity. The ESR does increase with increasing disease activity but this correlates more with colonic disease, and does not reflect the activity of small bowel disease (Fagan, Dyck et al. 1982, Dearing, McGuckin et al. 1969, Sachar, Smith et al. 1986, Sachar, Luppescu et al. 1990).

1.10.2.3 Orosomucoid

Orosomucoid (α1-acid glycoprotein) is another acute phase protein. As with CRP it is mainly produced by hepatocytes in response to potentially harmful stimuli (Desai, Faubion et al. 2007). Circulating levels of orosomucoid have been shown to correlate with disease activity in both CD and UC however; its 5 day half life means it cannot be reliably used as a measure of ongoing disease activity or response to treatment (Andre, Descos et al. 1981, Jensen, Jarnum et al. 1976).

1.10.3 IBD Serum Biomarkers

Anti-Saccharomyces cerevisiae antibodies (ASCA) and perinuclear antineutrophil cytoplasmic antibodies (P-ANCA) are autoantibodies to neutrophils and were the first extensively characterised serological IBD biomarkers (Sendid, Quinton et al. 1998, Satsangi, Landers et al. 1998).

1.10.3.1 ANCA

Anti-neutrophil cytoplasmic antibody (ANCA) tests were classically used in the diagnosis and monitoring of small vessel vasculitides (Papp, Norman et al. 2007). ANCA screening is carried out by indirect immunofluorescence (IIF) using ethanol-fixed neutrophils, as agreed in an international consensus (Savige, Gillis et al. 1999). IIF shows 2 major staining patterns: a cytoplasmic granular (C-ANCA) and perinuclear (P-ANCA) pattern. The C-ANCA pattern shows granular cytoplasmic fluorescence, frequently accentuated between the nuclear lobes. C-ANCAs are seen in the sera of patients with Wegner's granulomatosis and mainly recognise proteinase-3 (PR3) on enzyme-linked immunosorbent assays (ELISA) (Bossuyt 2006). The P-ANCA pattern results in a fine homogenous rim-like staining of the perinuclear cytoplasm. Nuclear extension can be present. P-ANCAs are present in patients with microscopic polyangiitis and recognise myeloperoxidase (MPO) on ELISA (Bossuyt 2006). It is recommended that ELISA should be performed on all serum samples since IFF alone detects only 90 – 95% of all ANCA positive samples (Savige J. Dimech W. Fritzler M. Goeken J. Hagen EC. Jennette JC. McEvoy R. Pusey C. Pollock W. Trevisin M. Wiik A. Wong R.

International Group for Consensus Statement on Testing and Reporting of Antineutrophil Cytoplasmic Antibodies (ANCA) 2003).

ANCAs have been identified in the sera of patients with chronic inflammatory disorders such as UC (40 – 80%) (Saxon, Shanahan et al. 1990, Rump, Scholmerich et al. 1990), primary sclerosing cholangitis (88%) (Terjung, Worman 2001), autoimmune hepatitis (81%) (Terjung, Bogsch et al. 2004) and CD (5 – 25%) (Bossuyt 2006) but in only 1 – 3% of healthy controls (Papp, Norman et al. 2007). In these disorders an atypical P-ANCA staining pattern is usually found and is recognised as a broad inhomogenous rim-like staining of the nuclear periphery often with multiple intranuclear foci (Terjung, Spengler et al. 2000).

1.10.3.2 ASCA

Anti-Saccharomyces cerevisiae antibodies (ASCAs) are antibodies primarily directed against a 200 kDa phosphopeptidomannan cell wall component of the common baker's or brewer's yeast *Saccharomyces cerevisiae* (Main, McKenzie et al. 1988). It is not clear, however, whether ASCA are truly directed against this particular yeast or whether they represent cross-reactivity with other, yet-unidentified microorganisms that also carry mannan sequences (Vermeire, Vermeulen et al. 2008). Both IgG and IgA antibodies are formed, and have been demonstrated in 50 – 80% of patients with CD, 2 – 14% of patients with UC and1 – 7 % of healthy individuals (Quinton, Sendid et al. 1998, Peeters, Joossens et al. 2001). Approximately two-thirds of CD patients with ASCA IgG are also positive for ASCA IgA, but 0 – 19% of the patients have only ASCA IgA antibodies, suggesting that both ASCA IgG and IgA should be measured. In CD, up to 90% specificity has been reported in specimens positive for both ASCA IgG and IgA antibodies, especially when the magnitude of both the IgG and IgA ASCA antibodies is high (Norman 2001). ASCA has the best combined sensitivity (31 – 45%) and specificity (90 – 100%) for diagnosing IBD, with a positive predictive value of 97 – 100% and a negative predictive value of 14 – 23% (Prideaux, De Cruz et al. 2012).

Combining these two tests may be of higher clinical value in the differentiation of CD and UC. The combined pattern of ASCA positive / P-ANCA negative for CD and ASCA negative / P-ANCA positive in UC has been shown to have a specificity of 81 – 98% (Quinton, Sendid et al. 1998, Peeters, Joossens et al. 2001, Sandborn, Loftus et al. 2001) however this increased sensitivity was associated with reduced sensitivity. In patients with IBD, positive predictive values of a positive ASCA test with a negative P-ANCA test for UC has been reported as ~93% (Quinton, Sendid et al. 1998, Koutroubakis, Petinaki et al. 2001).

1.10.3.3 Serological Biomarkers in IBDU/IC

Serological biomarkers have been used in an attempt to differentiate characterise IC. In a multicentre study (Joossens, Reinisch et al. 2002) of 97 patients with IC ASCA and P-ANCA was analysed. After 1 year a definitive diagnosis was reached in 31 patients. The combination of ASCA positive / P-ANCA negative predicted CD in 80% of IC patients (sensitivity 67%, specificity 78%). The combination of ASCA negative / P-ANCA positive was predictive of UC in 64% of the IC patients

(sensitivity78%, specificity 67%). Interestingly, 47 patients (48.5%) did not have antibodies to either ASCAs or ANCAs, and of these seronegative patients only 7 (14.9%) became UC or CD, compared with 24 of 50 (48%) seropositive patients. These seronegative patients may represent an as yet undefined clinicoserological subgroup of IBD and may express not yet specified antibodies (Guindi, Riddell 2004).

In the future wireless capsule endoscopy may provide another diagnostic tool for those with a diagnosis of IC, however whether the presence of small bowel lesions always represents a diagnosis of CD remains to be proven (Geboes, Colombel et al. 2008).

At present the use of the term "indeterminate colitis" has evolved from one based on histological examination of colectomy specimens to one that includes patients suspected of having IBD based on biopsy specimens without features enabling a clear diagnosis. The Montreal Working Group (Silverberg, Satsangi et al. 2005) have recommended that the term "indeterminate colitis" is applied only to resected specimens, and that "inflammatory bowel disease unclassified" (IBDU) should be used in all other cases. The reason for this is that the original term, IC, was proposed for colectomy specimens and that not all diagnostic features can be identified on partial thickness biopsy specimens. Consequently, patients who appear to have IBD colitis but who cannot be readily classified when all clinical, radiological, endoscopic, histologic, and serologic data are taken into account should be classified as IBDU.

Working towards biomarkers that can help in the classification of IC is vital for the future management of this condition.

1.10.3.4 Markers of Disease Activity

ASCA and P-ANCA have been studied with regard to developing a marker of disease activity or remission. In UC there has been shown to be no relationship between disease activity and the presence or titre of P-ANCA (Vasiliauskas, Plevy et al. 1996, Reumaux, Colombel et al. 2000), in fact the P-ANCA titre has been shown to remain positive even after colectomy (Reumaux, Colombel et al. 2000). In CD, the presence of ASCA has been shown to remain stable over time and is independent of activity, duration and usually remains stable after treatment (Vasiliauskas, Plevy et al. 1996, Vermeire, Peeters et al. 2001).

Neither alone nor in combination do these biomarkers translate into clinical practice for the diagnosis or monitoring of IBD. They may simply be used as adjuncts to potentially aid in the diagnosis of complex indeterminate cases.

A further 3 serological biomarkers were introduced to aim to solve this problem. These encompassed antibodies against the outer membrane porin C transport protein of *Escherichia coli* (anti-OmpC), *Pseudomonas fluorescens* bacterial sequence I2 (anti-I2), and bacterial flagellin (anti-CBir 1).

1.10.3.4.1 Anti-OmpC

Omp-C is a major outer membrane protein for which an excessive secretion of antibodies has been identified in IBD (Cohavy, Bruckner et al. 2000). The detection of IgA and IgG is carried out with

ELISA, and anti-ompC has been reported in 37 – 55% (Arnott, Landers et al. 2004, Landers, Cohavy et al. 2002) of CD patients. However, in children and young adults it appears to be less prevalent, only being identified in 24% of CD patients (Zholudev, Zurakowski et al. 2004).

Anti-ompC has been identified 2 – 11% (Zholudev, Zurakowski et al. 2004, Peyrin-Biroulet, Standaert-Vitse et al. 2007) of UC patients. In children and young adults it has been reported in 11% (Zholudev, Zurakowski et al. 2004) of cases.

In indeterminate colitis anti-ompC has been identified in 17 – 36% (Joossens, Colombel et al. 2006, Hui, Landers et al. 2005) of patients, and in 2 – 5% (Zholudev, Zurakowski et al. 2004, Peyrin-Biroulet, Standaert-Vitse et al. 2007) of healthy controls.

Mow et al (Mow, Vasiliauskas et al. 2004) found that patients expressing anti-ompC were significantly more likely to have internal perforating disease and require small bowel surgery than those in whom anti-ompC was negative.

1.10.3.4.2 Anti-I2

Microbical sequence I2 has been isolated in lamina propria mononuclear cells in colonic mucosa affected by CD (Sutton, Kim et al. 2000). This sequence derives from *Pseudomonas fluorescens* (Wei, Huang et al. 2002). Sutton et al (Sutton, Kim et al. 2000) identified IgA anti-I2 antibodies in 54% of people with CD, 10% of people with UC, 19% of people with other inflammatory enteric diseases (infectious colitis, radiation proctitis, *Shigella* colitis, eosinophilic colitis and collagenous colitis), and 4% of healthy controls. Joossens et al (Joossens, Colombel et al. 2006) found the prevalence of anti-I2 to be 41.9% in IC, 17.2% in healthy controls and 31.3% in IBD patients.

Patients with CD expressing anti-I2 are significantly more likely to have fibrostenosing disease and require small bowel surgery than those who are seronegative for anti-I2 (Mow, Vasiliauskas et al. 2004).

1.10.3.4.3 Anti-CBir1

Using serologic expression cloning, Cbir1 has been identified as an immunodominant and colitogenic antigen of the enteric microbial flora in C3H/HeJBir mice strain. Cbir1 is closely related to flagellin from *Butyrivibrio, Roseburia, Thermagota*, and *Clostridium* species, and appears in the *Clostridium* subphylum cluster XIVa of Gram-positive bacteria. Flagellins are molecules known to activate innate immunity via Toll-like receptor 5 (TLR5), and critical targets of the acquired immune system in host defence (Lodes, Cong et al. 2004).

Targan et al (Targan, Landers et al. 2005) showed that 50% of patients with CD will express anti-CBir1, compared with 6% of patients with UC, 8% of healthy individuals and 14% of patients with other inflammatory gastrointestinal diseases.

Anti-CBir1 has been shown to be an independent marker of CD. It has no correlation with disease activity and usually remains stable following surgery or anti-TNFα therapy. It is associated with small bowel, internal-penetrating and fibro-stenosing disease features, but not with small bowel surgery (Targan, Landers et al. 2005).

1.10.3.5 Anti-glycan Antibodies

Glycans are the predominant surface components of cells. They can be found on erythrocytes, immune cells, and microorganisms (Dotan, Fishman et al. 2006). They generate high levels of antiglycan antibodies of all classes, which have been demonstrated in a number of inflammatory and autoimmune diseases (Buonomano, Tinguely et al. 1999, McMorrow, Comrack et al. 1997, Willison, Yuki 2002).

Dotan et al (Dotan, Fishman et al. 2006) investigated anti-glycan antibodies as markers of IBD. Initially, in the discovery phase, mono-, di-, and trisaccharides were used to characterise the anti-glycan antibody profile in serum samples of patients with CD and UC, and in healthy controls. In the validation phase samples from IBD patients and healthy controls were screened to detect the presence of characteristic anti-glycan antibodies. After profiling anti-glycan antibodies by the glycan array using indirect immunofluorescence, an ELISA was established to determine the most discriminatory antibodies. These were gASCA, anti-Glc(β1,3)Glc(β) (laminaribioside) IgG and anti-GlcNAc(β 1,4)GlcNAc(β) (chitobioside) IgA. These novel antibodies were designated anti-laminaribioside carbohydrate IgG antibody (ALCA), and anti-chitobioside IgA carbohydrate antibody (ACCA). gASCA is similar to conventional ASCA but is a measure of antibodies against mannan (IgG anti-covalently attached anti-*Saccharomyces cerevisiae* antibodies).

In CD, ALCA and ACCA were positive in 37.5% and 36% of cases, compared to gASCA, which was positive in 65% of the discovery phase. In the validation phase ALCA, ACCA and gASCA were positive in 27%, 25% and 69% of CD patients; in 4%, 5% and 7% of UC patients; in 9%, 9% and 13% of the non-IBD gastrointestinal diseases group; and in 2%, 12% and 15% of healthy controls, respectively.

Anti-mannobioside (Man(α1,3)Man(α)) carbohydrate IgG antibody (AMCA) is another anti-glycan antibody that has been investigated in the setting of IBD biomarkers. Malickova et al (Malickova, Lakatos et al. 2010) measured gASCA, ACCA, ALCA and AMCA in patients with CD, UC and in healthy controls to determine the role of these markers in IBD. They found that in CD patients 67% were positive for gASCA, 27% for ACCA, 25% for ALCA and 31% for AMCA. In the UC group 14%, 45%, 22% and 36% were positive respectively. In the healthy control group 5.5% were positive to gASCA, 5.5% to ALCA, 11% to ACCA and 11% to AMCA.

It was identified that the combination of gASCA, ACCA, ALCA and AMCA did not aid differentiation of CD from UC when compared to gASCA alone. The combination did however aid differentiation of CD from healthy controls, and sensitivity of immunological diagnosis of CD was 85.5% when the markers were used in combination.

In this study, ACCA, ALCA and AMCA levels did not vary with disease duration and were not associated with disease behaviour or location; however Ferrante et al (Ferrante, Henckaerts et al. 2007) identified that ALCA is more often positive in the penetrating form of CD and ACCA in the stenosing form.

Malickova et al (Malickova, Lakatos et al. 2010) found that over half (56.4%) of gASCA negative CD patients are seropositive for ACCA, ALCA or AMCA. This is in keeping with Ferrante et al (Ferrante,

Henckaerts et al. 2007) who identified anti-carbohydrate antibodies in 32% of gASCA negative CD patients, and allowed Malickova et al to formulate the hypothesis that ASCA negative CD patients may represent a distinct immunological phenotypic group compared to those with ASCA positivity (Malickova, Lakatos et al. 2010).

Reider et al (Rieder, Schleder et al. 2010) evaluated a further two anti-glycan antibodies as novel markers in IBD; anti-laminarin IgA (Glc(β 1,3))3n(Glc(β 1,6))n carbohydrate antibody (Anti-L) and anti-chitin GlcNAc(β1,4)n IgA carbohydrate antibody (Anti-C). These were measured in conjunction with ACCA, ALCA, AMCA and gASCA.

They found that the combination of gASCA/P-ANCA was the most accurate for the diagnosis of CD versus UC. The addition of anti-L and anti-C to gASCA significantly increased the discriminatory capacity for CD versus no CD compared to gASCA alone, and the addition of anti-L and anti-C to the combination of gASCA/P-ANCA significantly increased the discriminatory capacity for CD versus UC, compared to gASCA/P-ANCA alone.

Overall, Reider et al (Rieder, Schleder et al. 2010) found that there was a poor correlation between the levels of the two new antibodies (anti-L and anti-C) and the established antibodies (ACCA, ALCA, AMCA and gASCA) suggesting distinct and independent serological responses. They did identify that anti-L had the strongest association with complications related to CD, and that anti-L and anti-C were strongly associated with the requirement for surgery. When multivariate analyses were carried out taking into account age, sex, BMI, disease location, age at time of diagnosis, and disease duration an independent association of gASCA, AMCA and anti-L was found with complicated CD behaviour, and gASCA, AMCA, anti-L and anti-C with the need for surgery. Patients with positivity for at least one serum marker had a higher likelihood of complicated disease behaviour, IBD-related surgery, early disease onset, and ileal and small bowel disease location compared to patients negative for all markers. Positivity for an increasing number of antibodies raised the likelihood for the occurrence of complicated disease behaviour, IBD-related surgery, early disease onset, and ileal and small bowel involvement.

These findings are in keeping with Seow et al (Seow, Stempak et al. 2009) who had also found that that adding anti-L and anti-C to gASCA/P-ANCA improved the differentiation between CD and UC. They also identified that adding anti-L to gASCA/P-ANCA differentiated colonic CD from UC, and that anti-L was associated with ileocolic CD. Seow et al (Seow, Stempak et al. 2009) also agreed with Rieder (Rieder, Schleder et al. 2010), Ferrante (Ferrante, Henckaerts et al. 2007) and Mow (Mow, Vasiliauskas et al. 2004), stating that an increasing number of positive antibodies is significantly ($p<0.001$) associated with an aggressive form of CD with early onset, penetrating phenotype, perianal disease and requirement for surgery.

1.10.3.6 Anti-UBE4A

Ubiquitination factor E4A (UBE4A) is a member of the of the U-box ubiquitin ligase family. The encoded protein is involved in multiubiquitin chain assembly and plays a critical role in chromosome condensation and separation through the polyubiquitination of securin, as well as cell cycle regulation,

cellular signaling in response to stress and to extracellular signals, morphogenesis, secretory pathways, DNA repair, and organelle biogenesis (Weissman 2001, Hershko, Ciechanover 1998). Anti-UBE4A levels have been shown to be significantly correlated with the Crohn's Disease Activity Index, as well as requirement of surgery and a stricturing or penetrating phenotype (Sakiyama, Fujita et al. 2008).

1.10.3.7 Pancreatic Autoantibodies

Pancreatic autoantibodies (PABs) have been considered as potential biomarkers in CD, since the 1980s (Stocker, Otte et al. 1987). However, attempts to define the target of these antibodies have proved challenging (Stocker, Otte et al. 1987, Seibold, Weber et al. 1991, Fricke, Birkhofer et al. 1999). Recently, Roggenbuck et al (Roggenbuck, Hausdorf et al. 2009) have shown that glycoprotein 2 (GP2), the major zymogen granule membrane glycoprotein, is the major autoantigen of PABs expressed at mRNA and protein levels in colonic biopsies from patients with CD. Previously in animal models, GP2 has been associated with the cholesterol–glycosphingolipid-enriched microdomains (lipid rafts) of secretory granule membranes of the pancreatic acinar cells (Schmidt, Schrader et al. 2001), has been found in lipid rafts of the brush-border membrane of small intestinal enterocytes (Nguyen, Amine et al. 2006), and has been shown to be specifically synthesised in murine M cells of Peyer's patches (Terahara, Yoshida et al. 2008). PAB-positivity (type I and type II) can be identified on the basis of indirect immunofluorescence (IIF) patterns related to the location of specific IIF signals (Seibold, Mork et al. 1997). Type II does not seem to differentiate CD patients from UC patients whereas type I PAB appears to do so (Joossens, Vermeire et al. 2004).

Anti-GP2 reactivity is not found in all PAB-positive patients thus revealing the potential for further autoantigenic PAB targets (Roggenbuck, Hausdorf et al. 2009, Roggenbuck, Reinhold et al. 2011, Roggenbuck, Bogdanos et al. 2013). Further investigation of these potential biomarkers may aid not only our diagnosis but also our understanding of CD.

1.10.3.8 CXCL16

CXCL16 is a transmembrane protein. It comprises an extracellular chemokine domain fused to a mucin stalk that extends through the cell membrane (Matloubian, David et al. 2000). CXCL16 is primarily expressed by macrophages (Wilbanks, Zondlo et al. 2001), dendritic cells and vascular smooth muscle cells (Hofnagel, Luechtenborg et al. 2002, Wagsater, Olofsson et al. 2004) in various lymphoid tissues including the thymus, spleen, lymph nodes and Peyer's patches, and in non-lymphoid tissues including the lung, liver, kidney and small intestine, but not colonic tissue (Matloubian, David et al. 2000, Shimaoka, Kume et al. 2000). It combines the functionality of a pattern recognition receptor with the properties of an inflammatory chemokine (Matloubian, David et al. 2000).

Lehrke et al (Lehrke, Konrad et al. 2008) investigated the association of CXCL16 with IBD and found that soluble serum levels of CXCL16 were significantly higher in patients with IBD than in healthy controls, although more so in CD than in UC. Levels were not found to be associated with disease

activity, and have been shown to be raised in other inflammatory disease processes (van der Voort, van Lieshout et al. 2005, Lehrke, Millington et al. 2007) thus potentially limiting the use of CXCL16 as a biomarker. However, more recently Uza et al (Uza, Nakase et al. 2011) have shown soluble serum levels of CXCL16 correlate with disease activity in CD and UC, as well as being significantly raised in healthy controls compared to IBD patients. CXCL16 was also shown to have a critical role in the progression of colonic inflammation. Further investigation is clearly required.

1.10.3.9 Apolipoprotein A-IV

Apolipoprotein A-IV, expresed in the small intestine, is a component of intestine-derived, triglyceride-rich lipoproteins (Apfelbaum, Davidson et al. 1987). It is secreted on lymph chylomicrons and was initially thought to have roles in lipid transport and lipoprotein metabolism (Goldberg, Scheraldi et al. 1990), control of food intake (Fujimoto, Fukagawa et al. 1993) and gastric function (Okumura, Fukagawa et al. 1994), although these have not been proven in knock-out mice (Weinstock, Bisgaier et al. 1997). It has however been shown to have anti-atherogenic, anti-oxidant and anti-inflammatory properties (Ostos, Conconi et al. 2001, Li, Conklin et al. 2008). Vowinkel et al showed apolipoprotein A-IV to inhibit DSS-induced colitis in mice (Vowinkel, Mori et al. 2004). Following this investigation of apolipoprotein A-IV levels in human IBD patients was investigated. Broedl et al identified that apolipoprotein A-IV levels were inversely related to CRP and disease activity in CD but not in UC (Broedl, Schachinger et al. 2007). This has so far not been taken further with regards a biomarker or treatment discovery.

1.10.3.10 Serum Calprotectin

Faecal calprotectin is well established as a marker of disease activity in IBD (Desai, Faubion et al. 2007), as well as having the potential to be used as a screening tool in the initial phase of investigation for IBD (van Rheenen, Van de Vijver et al. 2010).

Serum calprotectin is a calcium- and zinc-binding protein complex composed of 8 and 14 kD subunits S100A8 and S100A9. It is also known as calgranulin A/B, S100A8/A9, MRP8/14 or 27E10 antigen.

Calprotectin is mainly produced by neutrophils but is also present in macrophages. It is estimated to represent >40% and 5% of cytosolic and total proteins of neutrophils respectively (Yui, Nakatani et al. 2003).

The use of serum calprotectin as a biomarker has been investigated in other diseases such as cystic fibrosis (Gray, Imrie et al. 2010), diabetes mellitus type II (Ortega, Sabater et al. 2012), appendicitis (Kharbanda, Rai et al. 2012, Mills, Huckins et al. 2012), gynaecological cancers (Kostakis, Cholidou et al. 2010), and rheumatoid arthristis (Andres Cerezo, Mann et al. 2011) as it is a marker of inflammation.

In IBD, Lugering et al showed that serum levels of MRP8/14 (calprotectin) are elevated in both CD (Lugering, Stoll et al. 1995a) and UC (Lugering, Stoll et al. 1995b). More recently serum calprotectin has been shown to be elevated in children at diagnosis of IBD, and to correlate with paediatric disease activity scores (Leach, Yang et al. 2007), and Malíčková et al agreed with these findings and

themselves went on to show that serum calprotectin is significantly elevated in IBD patients prior to biological therapy, but in responders to therapy significantly reduces over a ten week period (Malickova, Kalousova et al. 2010).

1.10.4 Faecal Biomarkers in IBD

Faecal biomarkers have been strongly considered in IBD for a number of reasons. The faecal stream is in direct contact with the gut mucosa and therefore should contain specific markers of mucosal disease. The histological features of CD and UC include leucocyte infiltration into the gut wall and subsequent sloughing of the cells and their products into the bowel lumen and thus into the faecal stream. This is also associated with increased gut permeability and loss of the normal barrier function of the mucosa. As a result of these processes, potential faecal biomarkers include the faecal excretion of leucocytes, leucocyte products and serum proteins (Sutherland, Gearry et al. 2008).

Several candidate leucocyte-derived proteins have been identified, including faecal calprotectin, lactoferrin, myeloperoxidase, lysozyme, elastase, eosinophil cationic protein (ECP), eosinophil protein X (EPX), and human neutrophil lipocalin (HNL) (Klass, Neale 1978, Langhorst, Elsenbruch et al. 2005, Saiki 1998, Adeyemi, Hodgson 1992, van der Sluys Veer, Brouwer et al. 1998, Silberer, Kuppers et al. 2005, Peterson, Eklund et al. 2002).

1.10.4.1 ^{111}Indium-labelled Leucocytes

The faecal excretion of leucocytes is increased in IBD. The accurate measurement of excretion requires a radioactive-labelling method rather than a simple single faecal sample and is typically combined with abdominal scintigraphy to identify inflamed segments of intestine. The technique was initially described in 1981 (Segal, Ensell et al. 1981) using mixed leucocytes and since has been refined to use pure granulocyte preparations (Saverymuttu, Camilleri et al. 1986).

The faecal excretion of ^{111}Indium-labelled leucocytes is considered the gold standard faecal marker of inflammation since strict correlations with intestinal inflammation were evidenced at colonoscopy and histology (Angriman, Scarpa et al. 2007). Saverymuttu et al (Saverymuttu, Peters et al. 1985) reported a sensitivity of 97% for faecal ^{111}Indium-labelled leucocyte excretion compared with 79% for radiology and 70% for rectal histology in the diagnosis of IBD. However, this technique has a number of limitations; specialist facilities are required to for abdominal scintigraphy and to carry out sterile labelling of leucocytes, patients are exposed to a dose of radiation (8.5–17 millisieverts (mSv)), and the cost of this investigation is ~ £300 / patient. The other drawback of this technique is that it requires a 4 day collection of faecal material, and the practicalities of this are somewhat limiting. It has therefore remained essentially a tool used in research, rather than translating into clinical practice (Tibble, Teahon et al. 2000).

1.10.4.2 ^{51}Chromium-labelled Red Cells

Teahon et al (Teahon, Bjarnason 1993) compared the faecal excretion of ^{51}Chromium-labelled red cells with ^{111}Indium-labelled leucocytes in patients with IBD. Intestinal inflammation and blood loss were significantly correlated in patients with UC, but not in those with CD.

As with measuring the faecal excretion of ^{111}Indium-labelled leucocytes, this test is relatively expensive, involves an exposure to radiation, and a four day faecal collection, all of which make it a suboptimal faecal marker.

1.10.4.3 Faecal Calprotectin

Calprotectin is a heterocomplex protein consisting of 2 heavy (L1H) chains and 1 light (L1L) chain which are non-covalently linked (Bjerke, Halstensen et al. 1993). It was initially known as protein L1 (Fagerhol, Dale et al. 1980) and is a calcium-binding protein that exhibits antibacterial and antifungal activity, inhibits metalloproteinases, and induces apoptosis in malignant and non-malignant cell cultures (Steinbakk, Naess-Andresen et al. 1990). It has been found to represent approximately 60% of neutrophilic cytosolic protein (Fagerhol, Dale et al. 1980, Roseth, Fagerhol et al. 1992), is released from cells during cell activation or death (Desai, Faubion et al. 2007), and is stable in faeces for up to one week at room temperature (Roseth, Fagerhol et al. 1992).

Faecal calprotectin (FC) is the most commonly used faecal biomarker in IBD, and can be quantified using ELISA (Desai, Faubion et al. 2007). Single random stool samples of <5 g show FC concentrations equivalent to 24-hour homogenised specimens, indicating that FC is evenly distributed through the faeces (Roseth, Fagerhol et al. 1992). These qualities increase the clinical utility of FC, allowing for potential delays in performing the assay, transport to a central laboratory, and the analysis of small samples (Sutherland, Gearry et al. 2008).

The median FC level is 2 mg/L in healthy individuals with an upper limit of 10 mg/L (Desai, Faubion et al. 2007). In 2000, Ton et al (Ton, Brandsnes et al. 2000) developed an improved assay to measure FC. Since then the units have been changed to be measured in µg/g rather than mg/L. In order to convert from the older assays to the newer, figures need to be multiplied by a factor of 5.

FC level has been shown to be a sensitive marker of activity in CD and to correlate well with endoscopic and histological activity in UC (Tibble, Teahon et al. 2000, Roseth, Fagerhol et al. 1992, Roseth, Aadland et al. 1997). Tibble et al also showed that FC normalises with endoscopic healing in CD (Tibble, Teahon et al. 2000).

Roseth et al (Roseth, Schmidt et al. 1999) have shown that FC measurement correlates well with the more difficult and more expensive measurement of ^{111}Indium-labelled leucocyte excretion in IBD.

A recent meta-analysis (van Rheenen, Van de Vijver et al. 2010) has shown that FC is a useful screening tool in the identification of those who require endoscopic investigation for suspected IBD. This test however appears to be more useful in an adult population that in children or adolescents.

1.10.4.4 Faecal Lactoferrin

Lactoferrin is an iron-binding protein and a major component of neutrophil secondary granules. It is secreted into the local environment upon neutrophil degranulation and can be found in many body

fluids (Buderus, Boone et al. 2004). During intestinal inflammation, polymorphonuclear neutrophils infiltrate the mucosa, resulting in an increase in the concentration of faecal lactoferrin (FL). Its presence is proportional to neutrophil translocation to the gastrointestinal tract (Guerrant, Araujo et al. 1992). The protein is resistant to proteolysis and undamaged by multiple freeze thaws, therefore providing a useful marker in faeces as an indicator of intestinal inflammation (Angriman, Scarpa et al. 2007).

A commercial ELISA has been developed for lactoferrin and is now widely available. It has been used extensively in the investigation of infectious diarrhoea and is highly sensitive for detecting faecal neutrophil infiltration (Sutherland, Gearry et al. 2008).

The concentration of FL in healthy individuals is 1.45 ± 0.4 µg/g of faecal weight. In active IBD this value can rise to several hundred µg/g of faecal weight (Kane, Sandborn et al. 2003). FL is increased in patients with active IBD when compared to those with inactive IBD with specificity between 85% and 90%, and levels may rise significantly prior to a clinically evident relapse (van der Sluys Veer, Biemond et al. 1999, Walker, Land et al. 2004).

Buderus et al (Buderus, Boone et al. 2004) reported on a study of five paediatric patients with CD undergoing infliximab therapy. FL levels were determined before and after infliximab treatment. It was determined that FL rapidly decreased after clinically successful treatment with infliximab in children with severe CD. They stated that FL was a sensitive and specific marker for intestinal inflammation that can be used for monitoring the therapeutic response to infliximab in paediatric patients with CD.

Parsi et al (Parsi, Shen et al. 2004) studied patients with an IPAA. The aim was to use FL to determine whether symptomatic patients had pouchitis, cuffitis or CD, or whether they had a non-inflammatory condition such as irritable pouch syndrome. Faecal samples were taken and analysed for FL, and these results were correlated with endoscopy / biopsy results. It was observed that symptomatic patients with an inflammatory condition had significantly higher FL concentrations (median 176.0 µg/mL) than those with a non-inflammatory condition (median 4.8 µg/mL), or those who were asymptomatic (median 7.8 µg/mL), $p<0.001$. At a cut-off level of 7 µg/mL, FL could distinguish patients with irritable pouch syndrome from those with pouchitis, cuffitis, or CD with a sensitivity of 100% and specificity of 85%. Therefore this group felt that FL can serve as a sensitive and non-invasive initial screening test in the evaluation of symptomatic patients after restorative proctocolectomy for UC.

Recent studies comparing FL and FC have suggested that both tests are similarly useful in the assessment of the disease activity of IBD. Faecal excretion of calprotectin has been shown to correlate with findings of colonic inflammation at endoscopic examination, whilst faecal excretion of lactoferrin correlates with histological inflammation (Langhorst, Elsenbruch et al. 2005, D'Inca, Dal Pont et al. 2007).

Reference ranges between active and quiescent IBD for FL overlap. This restricts its use to that of diagnosing new cases of IBD, and despite the early promise of FL there have been limited further studies on its use in this area (Poullis, Foster et al. 2002).

1.10.4.5 Faecal Lysozyme

Lysozyme, also known as muramidase, is a polymorphonuclear neutrophil-derived enzyme which catalyses the hydrolysis of Gram-positive bacterial cell walls (Angriman, Scarpa et al. 2007). Faecal lysozyme is elevated in patients with inactive and active CD, active UC, and non-inflammatory gastrointestinal diseases with diarrhoea, when compared to healthy controls (Klass, Neale 1978, van der Sluys Veer, Brouwer et al. 1998). It has been shown to correlate with excretion of ^{111}Indium-labelled leucocytes granulocytes in patients with colonic disease but not in those with small bowel disease (Crama-Bohbouth, Pena 1998). None of these factors make this a particularly useful biomarker in clinical practice, and faecal lysozyme has in fact been shown to be less effective than other markers in the diagnosis of IBD (Langhorst, Elsenbruch et al. 2005, Silberer, Kuppers et al. 2005).

1.10.4.6 Faecal Myeloperoxidase

Myeloperoxidase is a constituent of neutrophil azure granules (Angriman, Scarpa et al. 2007). It is released during inflammation and plays a role in the oxygen-dependent killing of micro-organisms and tumour cells. Its concentration within a suspension has been found to be proportional to the number of neutrophils within that suspension (Saiki 1998, Peterson, Eklund et al. 2002, Sugi, Saitoh et al. 1996).

Faecal levels of myeloperoxidase are elevated in active IBD compared with controls and correlate with laboratory parameters and endoscopic grade of inflammation (Saiki 1998), however this correlation has been shown to be less efficient that FL in the diagnosis of IBD (Silberer, Kuppers et al. 2005). This may be due to the shorter half life of myeloperoxidase, but it means that it has not been established as a clinically useful faecal biomarker.

1.10.4.7 Polymorphonuclear Neutrophil Elastase

Polymorphonuclear Neutrophil Elastase (PMN-E) can be identified in the faeces of patients with IBD. It can be measured as either a complex bound to α1-antitrypsin (α1-AT), or as free faecal elastase (Adeyemi, Hodgson 1992, Sugi, Saitoh et al. 1996). PMN-E has been shown to correlate with the Crohn's Disease Activity Index (CDAI) and a numerical disease activity index for UC (Adeyemi, Hodgson 1992). However, PMN-E, when compared directly with FL, is an inferior biomarker in IBD (Langhorst, Elsenbruch et al. 2005, Sugi, Saitoh et al. 1996).

1.10.4.8 Eosinophil Derived Protein

In patients with a variety of intestinal diseases, including IBD, increased eosinophil levels and enhanced eosinophil activation have been seen in the intestinal mucosa (Heatley, James 1979, Talley, Kephart et al. 1992, Bischoff, Wedemeyer et al. 1996). The specific granules of the eosinophils contain a number of highly cationic proteins including ECP and EPX. These proteins have potent cytotoxic action and immunomodulatory properties, and are released from the cells after activation and stimulation of the cells (Gleich, Adolphson 1986).

The eosinophil may also be a major factor in the pathogenesis of IBD because biopsies from these patients have demonstrated an infiltration of eosinophils in the lamina propria (Bischoff, Wedemeyer et al. 1996, Sarin, Malhotra et al. 1987, Makiyama, Kanzaki et al. 1995, Oshitani, Sawa et al. 1997, Raab, Hallgren et al. 1992) and marked extracellular deposits of ECP (Raab, Hallgren et al. 1992). There is also an excess release of the eosinophil proteins ECP and EPX in luminal fluid and feces of patients with active UC or CD (Berstad, Borkje et al. 1993, Bischoff, Grabowsky et al. 1997, Levy, Gleich et al. 1997, Saitoh, Kojima et al. 1999).

ECP and EPX can be measured by radioimmunoassay, and are stable in faeces for at least three days when stored at 22°C (Peterson, Eklund et al. 2002), a key element for a useful biomarker. Saitoh et al (Saitoh, Kojima et al. 1999) found EPX to be more stable than ECP when stored at 25°C for 48 hours, and therefore postulated that EPX is a better marker.

Peterson et al (Peterson, Eklund et al. 2002) showed that both ECP and EPX are elevated in CD and to a greater extent in UC when compared to healthy individuals, and that there seems to be an association in CD between disease activity and EPX concentration, but less so for ECP. No association was shown between EPX or ECP and disease activity in UC.

Peterson's (Peterson, Eklund et al. 2002) findings agree with Saitoh et al (Saitoh, Kojima et al. 1999) who also showed that faecal ECP and EPX concentrations were significantly increased in both active UC and active CD compared to inactive UC and inactive CD, respectively. This study also reported that in the inactive phase CD patients who relapsed within 3 months had higher levels of ECP and EPX than those who remained in the inactive phase. Therefore Saitoh et al felt that ECP and EPX could be used as markers for evaluating disease activity and predicting relapse.

Currently there is no defined clinical role for the routine clinical use of faecal EPX or ECP in the diagnosis or monitoring of IBD (Sutherland, Gearry et al. 2008).

1.10.4.9 Human Neutrophil Lipocalin

Human neutrophil lipocalin (HNL) is a neutrophil granule protein which is stored within secondary granules (Xu, Carlson et al. 1994). It is a specific marker of neutrophils (Seveus, Amin et al. 1997). Peterson et al (Peterson, Eklund et al. 2002) observed that faecal HNL concentrations are markedly increased in UC and moderately increased in CD compared to healthy individuals, and that the levels in UC are significantly higher than those seen in CD ($p<0.0002$). A correlation between faecal HNL and disease activity was unable to be established.

1.10.4.10 Alpha 1-antitrypsin

Alpha 1-antitrypsin (α1-AT) is a protease inhibitor produced by the liver, macrophages, and the intestinal epithelium (Angriman, Scarpa et al. 2007). It is present in blood and is excreted in the faeces in increased concentrations in a number of disease states causing increased intestinal permeability and therefore protein loss (Meyers, Wolke et al. 1985). It has in fact been shown to be a good marker of protein losing enteropathy (Bernier, Florent et al. 1978).

α1-AT is relatively stable in faeces and can be measured using commercially available assays (van der Sluys Veer, Biemond et al. 1999, Wilson, McGilligan et al. 1988). Random stool samples can be used to measure faecal α1-antitrypsin concentration or alternatively α1-antitrypsin clearance can be calculated from a 24-hour faecal collection and measurement of total faecal α1-AT and serum α1-AT (Meyers, Wolke et al. 1985). Becker et al (Becker, Berger et al. 1999) showed that random faecal α1-AT levels are as useful as long collections in measuring CD activity, but the same is not true in UC patients.

Faecal excretion is increased in active CD and correlates well with clinical disease activity scores and the extent of small bowel disease in CD (Meyers, Wolke et al. 1985, Grill, Hillemeier et al. 1984). However, this is not a marker widely used in clinical practice as the method is not routinely available. There is also some controversy regarding conflicting evidence on the efficacy of faecal α1-AT as a diagnostic and monitoring tool in IBD (Angriman, Scarpa et al. 2007, Poullis, Foster et al. 2002). Furthermore, data about the efficiency in the follow-up after medical or surgical therapy, such as in case of pouchitis, are lacking (Parsi, Shen et al. 2004, Poullis, Foster et al. 2002) and other markers were demonstrated more accurate or cost-effective than faecal α1-AT in the assessment of patients with IBD (van der Sluys Veer, Biemond et al. 1999).

1.10.4.11 Alpha 2-macroglobulin

Alpha 2-macroglobulin (AMG) is a serum anti-proteinase (Angriman, Scarpa et al. 2007). In healthy subjects it is excreted in the faeces in small quantities (Poullis, Foster et al. 2002); however Becker et al (Becker, Niederau et al. 1999) have shown that in CD and UC it is present in elevated levels, and that in active disease it is far more prevalent than in quiescent disease. They also observed that faecal AMG correlated to the CDAI in subjects with CD but not to the CAI in subjects with UC. This method is not available in clinical practice (Angriman, Scarpa et al. 2007).

1.10.4.12 Tumour Necrosis Factor Alpha

Tumour necrosis factor alpha (TNF-α) is a proinflammatory cytokine produced by polymorphonuclear neutrophils. Braegger et al (Braegger, Nicholls et al. 1992) studied faecal TNF-α in children with IBD and found it to be a useful marker of disease activity. Kapel et al (Kapel, Roman et al. 2005) showed that the use of faecal TNF-α in diagnosing IBD was of equal value to the use of FC. Faecal TNF-α returns to normal levels in the quiescent disease phase or after curative surgery (Poullis, Foster et al. 2002).

Given that faecal TNF-α requires to be kept frozen where other faecal biomarkers do not (Peterson, Eklund et al. 2002), this technique does not translate well into clinical practice. Large inter-individual variations of levels in both serum and faeces have also limited also its routine use (Konikoff, Denson 2006), and it has not been fully evaluated in an adult population (Poullis, Foster et al. 2002).

1.10.4.13 Faecal Lactate

DL-Lactate is an intermediary metabolite in the bacterial fermentation of carbohydrates in the colon (Hove, Nordgaard-Andersen et al. 1994). The faecal concentration is normally less than 2-3 mmol/l (Mortensen, Hove et al. 1991). The combined large-bowel flora produces both stereoisomers, L- and D-lactate, which are further metabolised to shortchain fatty acids, the principal end-products of bacterial fermentation (Mortensen, Hove et al. 1991). In patients with IBD (Vernia, Caprilli et al. 1988, Vernia, Gnaedinger et al. 1988), as well as patients with severe malabsorption after extensive gastrointestinal resections (Oh, Phelps et al. 1979, Schoorel, Giesberts et al. 1980, Stolberg, Rolfe et al. 1982, Traube, Bock et al. 1983), an increased level of faecal lactate has been observed.

It is hypothesised that intestinal malabsorption increases the bacterial production of both D- and L-lactate from the non-digested luminal carbohydrates present in the contents of the large bowel (Hove, Nordgaard-Andersen et al. 1994). The production of intestinal lactate in inflammatory bowel diseases (IBD) may, however, derive from the anaerobicity of the inflamed mucosa, which probably forms the L-isomer, since D-lactate is not normally produced by mammalian cells (Christopher, Eckfeldt et al. 1990).

Hove et al (Hove, Nordgaard-Andersen et al. 1994) studied faecal DL-lactate in 100 patients with severe gastrointestinal diseases. It was observed that faecal L-lactate is elevated in IBD associated with proctitis, but not in quiescent IBD, IBD without proctitis or Crohn's ileitis. As proctitis is easily visible using rigid or flexible sigmoidoscopy, measuring faecal lactate does not add relevant clinical information (Poullis, Foster et al. 2002).

1.10.4.14 Platelet Activating Factor

Platelet activating factor (PAF) is a potent endogenous mediator in the inflammatory process (Poullis, Foster et al. 2002). It induces microvascular leakage, vasodilatation, smooth muscle contraction, and both platelet and leucocyte aggregation (Oshimoto, Okamura et al. 2005). Faecal PAF has been shown to correlate with the endoscopic extent of bowel inflammation in UC and CD, but not with disease activity scores or systemic inflammatory markers (CRP and ESR) (Hocke, Richter et al. 1999).

1.10.4.15 Cytokines

It is postulated that cytokines appear in the stool as they are produced locally in the diseased bowel and leak into the bowel lumen, or that IBD affected epithelial cells and immune-system cells, both capable of producing cytokines (Eckmann, Jung et al. 1993, Casini-Raggi, Kam et al. 1995), may be extruded into the bowel lumen and appear in the stool. The theory that the cytokines in the stool arise from leakage from the blood vessels within the gut wall is excluded by previous data showing 100-fold higher levels of various cytokines in stool than in plasma of patients with acute shigellosis (Raqib, Wretlind et al. 1995).

Saiki et al (Saiki, Mitsuyama et al. 1998) carried out a study to identify and quantify cytokines with pro- and anti-inflammatory properties in the stools of IBD patients. Stool concentrations of IL-1β and its natural antagonist IL-1ra (receptor antagonist) were significantly higher in patients with active IBD than in patients with inactive disease and normal controls. Stool concentrations of IL-4 and IL-10 in

active disease were not statistically different to those of patients with inactive disease or controls, as were TNF-α levels. In a paired analysis of the same individual, stool concentrations of TNF-α, IL-1β and IL-1ra were shown to decrease after the clinical resolution of disease exacerbation, and levels of IL-4 and IL-10 to increase. The reason for this is unclear. It is however conceivable that IL-4 and IL-10 may play a down-regulatory role in the acute inflammatory process.

1.10.4.16 Faecal S100A12

S100A12, is a calcium-binding protein, previously known as calgranulin C or EN-RAGE (extracellular newly identified receptor for advanced glycation end products). It is expressed as a cytoplasmatic protein in activated neutrophils with proinflammatory properties. The S100A12 activates the nuclear factor-κB signal transduction pathway and induces proinflammatory cytokine release, including TNF-α (Angriman, Scarpa et al. 2007). Elevated serum S100A12 levels are seen in inflammatory diseases such as rheumatoid arthritis (Marenholz, Heizmann et al. 2004, Foell, Kane et al. 2003) and cystic fibrosis (Foell, Seeliger et al. 2003), as well as in the serum and mucosa of children (Leach, Messina et al. 2004) with IBD and in the serum of adults with IBD (Foell, Kucharzik et al. 2003). De Jong et al (de Jong, Leach et al. 2006) studied in children and found that faecal S100A12 is distributed evenly in faeces and remains stable for 7 days, desirable characteristics for a potential biomarker. Faecal S100A12 was elevated in children at the time of diagnosis of IBD and could distinguish IBD children from normal healthy control subjects with 96% sensitivity and 92% specificity. Faecal S100A12 levels were shown to correlate closely with the PCDAI and standard serum inflammatory markers, specifically when there is lower gut involvement, and induction of clinical and biological remission in children leads to reduced faecal levels of S100A12.

It was proposed that at present faecal S100A12 could not replace colonoscopy and biopsy in the diagnosis of IBD in children, but that it may form a useful screening tool in deciding whether colonoscopy and biopsy is warranted. It may also be a useful non-invasive monitor of disease activity.

1.10.4.17 Nitric Oxide

Nitric oxide (NO) is an endogenously produced gas. In response to acute proinflammatory cytokines leucocytes and epithelial cells express iNOS leading to the production and accumulation of significant quantities of NO (Lundberg, Hellstrom et al. 2005).

Lundberg et al (Lundberg, Hellstrom et al. 1994) directly measured luminal NO in 12 controls and 6 patients with acute UC by a chemiluminescence technique. The gas was aspirated from different areas of the colon at colonoscopy. In the UC patients NO concentrations were found to be >100 times higher than in the controls.

Perner et al (Perner, Nordgaard et al. 2002) showed that in acute UC patients the production of NO in the colon is 10 times higher than controls ($p<0.001$), and that the results positively correlated with disease activity scores ($p<0.01$).

Although this technique can be carried out using a silicone catheter in the rectum to aspirate NO rather than having to use a colonoscope, it remains an invasive technique that is technically challenging and is not in routine clinical practice at present (Sutherland, Gearry et al. 2008).

1.11 Proteomics

The National Human Genome Research Institute and the US Department of Energy launched the human genome project in 1990 (National Human Genome Research, Institutes of Health et al. 2003). The objective was to sequence the entire human genetic code. From this the first CD gene (IBD1 locus on chromosome 16) was identified (Hugot, Laurent-Puig et al. 1996) and many others were to follow. However, this information does not elucidate the physiological processes ongoing during which millions of molecules resulting from an unknown number of post-translational modifications interact at varying time points (Yau, Leong et al. 2013).

Other molecular biology technologies therefore developed with the aim of understanding transcription and the resulting protein activity, and identifying physiological pathways. These have been termed "functional genomics", "systems biology" or "omics" encompassing genomics, transcriptomics, proteomics and metabolomics.

Proteomics has revolutionised the way in which biomarker discovery can be carried out as it allows large scale, high throughput identification and analysis of proteins in different biological fluids with the potential for elucidation of biological pathways affected in disease, the identification of individuals at high risk of disease, the assessment of prognosis and prediction of response to treatment, as well as the personalisation of therapeutic interventions and the prediction of those who may develop side-effects of a therapeutic intervention (Guest, Gottschalk et al. 2013).

1.11.1 Analytical Platforms for Proteomics

Mass spectrometry (MS) has become the method of choice for analysis of complex protein samples. MS-based proteomics is made possible by the availability of gene and genome sequence databases, and the development of protein ionisation methods.

A mass spectrometer consists of an ion source, a mass analyser that measures the mass-to-charge ratio (*m/z*) of the ionised analytes, and a detector that registers the number of ions at each mass spectrometric measurements are carried out in the gas phase on ionised analytes *m/z* value (Aebersold, Mann 2003).

Electrospray ionisation (ESI) ionises the analytes out of a solution. It is coupled with liquid-based separation tools such as chromatography or electrophoresis (Fenn, Mann et al. 1989).

Matrix-assisted laser desorption / ionisation (MALDI) sublimates and ionises the samples out of a dry, crystalline matrix via laser pulses (Karas, Hillenkamp 1988). MALDI-MS is usually utilised in the analysis of simple peptide mixtures whereas ESI-MS is preferred in the analysis of more complex samples (Aebersold, Mann 2003).

There are four basic types of mass analyser currently used in proteomics:

- Ion trap

- Time-of-flight (ToF)
- Quadropole
- Fourier transform ion cyclotron (FT)

These can be used as stand-alone analysers or in tandem to maximise the strengths of each.

Table 1.6: Advantages and Disadvantages of Mass Analysers (Aebersold, Mann 2003)

Platform	**Advantages**	**Disadvantages**
Ion trap	Robust, sensitive, inexpensive	Low mass accuracy
ToF	High sensitivity, high resolution, high mass accuracy, well suited for pulsed ionisation methods	Requires pulsed ionisation method or ion beam switching, limited dynamic range, limited precursor-ion selectivity
Quadropole	High sensitivity, high resolution, high mass accuracy, can be used with ESI ionisation source	Peak heights variable as a function of mass (mass discrimination), not well suited for pulsed ionisation methods
FT	High sensitivity, high mass accuracy, high resolution, dynamic range	Expensive, operational complexity, low peptide-fragmentation efficiency

High-resolution two dimensional gel electophoresis (2-DE) is used in combination with MS to allow the differentiation of all of the protein species. The commonly applied 2-DE methods separate the complex protein mixture by:

1. Charge separation employing isoelectic focusing (IEF)
    a. IPG (immobilised pH gradient) strips
    b. NEPHGE (Nonequilibrium pH Gel Electrophoresis) technique
2. Size separation involving SDS-PAGE (Sodium Dodecyl Sulfate – PolyAcrylamide Gel Electrophoresis) (Wittmann-Liebold, Graack et al. 2006)

1.11.2 Proteomics in IBD

In 2002 the first hypothesis-free proteomics research in IBD was published, and examined protein changes in human intestinal epithelial cells exposed to IL-γ, IL-1β, and IL-6. High levels of indoleamine-2,3-dioxygenase was found in IBD subjects compared with controls and the authors hypothesised an involvement of the kynurenine pathway of tryptophan metabolism in IBD (Barcelo-Batllori, Andre et al. 2002).

The first serum proteomic study of IBD was carried out in 2007 using surface-enhanced desorption ionisation (SELDI)-TOF MS for the initial proteome scan, followed by MALDI MS/MS, Western blotting and ELISA assay analysis of the proteins of interest. The profiles of subjects with CD, UC, nonspecific inflammation, and healthy controls were analysed and four biomarker candidates were validated (Meuwis, Fillet et al. 2007).

The follow-up to the previous study compared CD subjects before and after infliximab treatment, comparing the responeders with the non-responders. The previously identified candidate biomarker platelet factor 4 was found to be significantly higher in non-responders than in responders (Meuwis, Fillet et al. 2008).

More recently, Presley et al (Presley, Ye et al. 2012) have defined the metaproteome, protein expression on the mucosal-luminal interface of the intestine. The contents of this intestinal layer were analysed using SELDI-TOF MS and the results correlated bacterial phylotypes with specific immunological protein features. This work has the potential to discover host-microbe interactions in IBD pathogenesis.

1.11.3 Combining Proteomics and Metabolomics

The ability to explore both genetic and environmental factors in the pathogenesis of IBD is exciting. Proteomics and metabolomics offer the potential to study both endogenous and exogenous processes of the phenotypic manifestations of disease, and whilst these technologies are still in their relative infancies, they are progressing rapidly and are likely to offer valuable insights in the future.

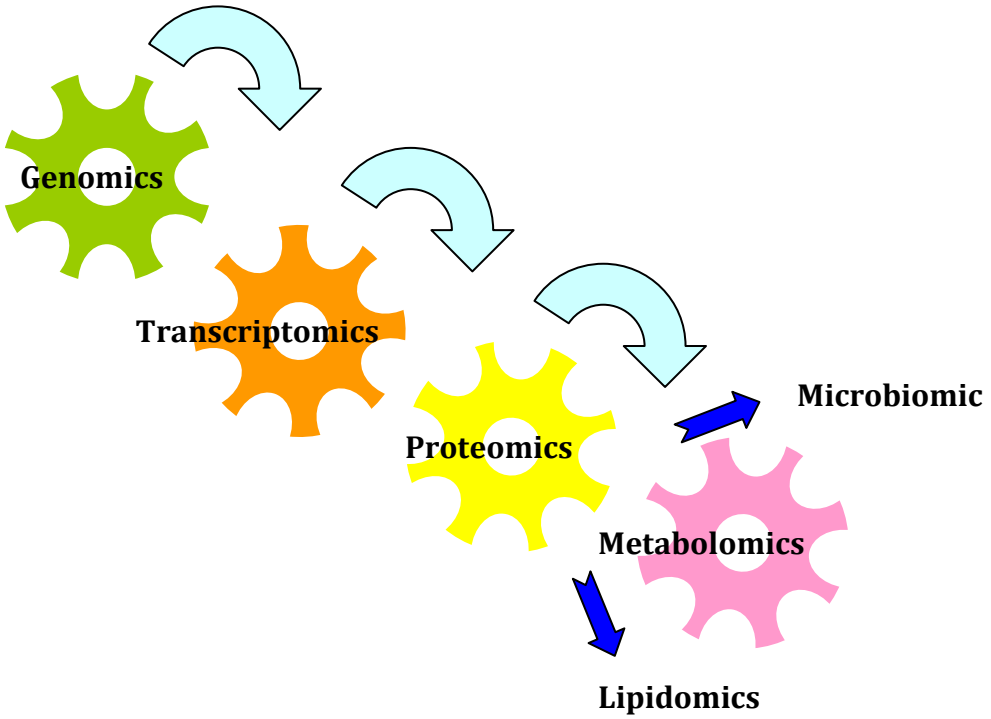
1.12 Metabolomics

The phrase "the metabolome" was coined in the late 1990s (Oliver, Winson et al. 1998, Tweeddale, Notley-McRobb et al. 1998) and was initially defined as the total quantitative collection of low molecular weight compounds present in a cell or organism which participate in metabolic reactions required for growth, maintenance and normal function (Oliver, Winson et al. 1998). Nicholson et al (Nicholson, Wilson 2003) went on to further clarify this definition as "the measurement of metabolite concentrations and fluxes and secretion in cells and tissues in which there is a direct connection between the genetic activity, protein activity and the metabolic activity itself".

The metabolome consists of thousands of organic and inorganic species, generally of weight < 1500 Daltons (Da) (Dunn 2008) or < 3000 *m/z* (Hollywood, Brison et al. 2006). The symbol *m/z* is used to denote the dimensionless quantity formed by dividing the mass of an ion in unified atomic mass units by its charge number, giving the mass-to-change ratio (McNaught, Wilkinson 1997).

The "omics" technologies primary aim is the non-targeted identification of all of the products of gene expression in order for us to understand the biological system as a whole. Information flow is from the genome via transcriptomics (the measurement of mRNA levels for quantifying gene expression) and proteomics (protein translation, including post-translational modifications) to metabolomics (Weckwerth 2003). The metabolome is the furthest "downstream" and is therefore can be thought of as the closest to the phenotype.

Figure 1.6: The "omics" cascade



Measuring the metabolites of patients with IBD is not a new concept. In 1980, Roediger and Truelove (Roediger, Truelove 1979) identified luminal *n*-butyrate as the major respiratory fuel of isolated colonocytes, and identified that colonocytes taken from patients with UC have an impaired ability to metabolise butyrate (Roediger 1980). In 1982, Roediger et al (Roediger, Heyworth et al. 1982) reported on the measurement of short chain fatty acids (SCFA), ammonia, sodium and potassium concentrations in the colon of patients with UC. They identified increased levels of luminal SCFA in patients with severe UC. In particular, butyrate levels were found to be increased in all UC patients, a finding independent of the disease severity. This confirmed that butyrate utilisation by the colonic mucosa is impaired in UC. It was thought that in the short term this deficiency lead to mucosal hypoplasia, and in the long term colitis (Scheppach, Christl et al. 1997). Therefore, SCFA enemas were used to attempt to reduce colonic inflammation, but were not shown to be successful (Breuer, Soergel et al. 1997, Scheppach 1996, Steinhart, Hiruki et al. 1996). This lead to the hypothesis that the inflammatory process is responsible for the decrease in colonic mucosal cell metabolism, or that the decrease in metabolism may be the result of a primary or acquired mucosal enzymatic defect, rather than unavailability of the mucosal substrate (Duffy, Regan et al. 1998). Duffy et al (Duffy, Regan et al. 1998) reported that mucosal metabolic fluxes of butyrate and glutamine are reduced in both UC and CD in comparison to healthy individuals. They showed that the differences were most marked in those with moderate to severe mucosal inflammation, and are not a result of a panenteric metabolic disorder in either condition, but simply a result of the inflammatory process. This is supported by Finnie et al (Finnie, Taylor et al. 1993) who found that patients with longstanding quiescent UC had no defect of butyrate metabolism.

The introduction of mass spectrometry in relation to metabolomic profiling has meant that vast numbers of metabolites can be identified from tissue, bodily fluids, plants and microbes. The mass spectrometer was first invented in 1912 (Dunn 2008) by J.J Thompson, and in 1922 Francis William Ashton won the Nobel Prize for Chemistry for his design and use of the mass spectrograph. Mass spectrometers operate by ion formation, separation of ions according to their mass-to-charge (*m/z*) ratio and detection of separated ions (Dunn, Ellis 2005).

In 2005, the Metabolomics Standards Initiative (MSI) was formed to oversee the standardisation of techniques in this rapidly expanding field. In 2001, a group from Imperial College London (Lindon, Nicholson et al. 2003) was working on metabonomic toxicity. This involved generating databases of ^{1}H NMR spectra of body fluids from animals. Six major pharmaceutical corporations were involved and due to the collaborative nature of the project standards were needed to exchange data and communicate results. The Standard Metabolic Reporting Structure (SMRS) initiative was formed at this time and reported in 2005 (Lindon, Nicholson et al. 2005). Simultaneously a consortium focused on the metabolomic profiling of plants was working on a generic data model to provide a basis for the design of systems for data storage and exchange, called Architecture for Metabolomics (ArMet) (Jenkins, Hardy et al. 2004). These two groups merged to form the MSI (Castle, Fiehn et al. 2006) and aim to provide a common mechanism for describing the work so that the data can be made available to others for evaluation, or to support an extension or repeat of the work as desired, or published in a public repository (Fiehn, Robertson et al. 2007).

The metabolome has been studied in relation to microbes (Khoo, Al-Rubeai 2007, Koek, Muilwijk et al. 2006, Mashego, Rumbold et al. 2007), plants (Kopka, Fernie et al. 2004, Hall 2006), fruits (Biais, Allwood et al. 2009), the environment (Lim, Viant et al. 2006, Viant 2007), and mammals (Sellick, Hansen et al. 2009). With regards to the medical world the study of health and nutrition (Fava, Lovegrove et al. 2006, German, Watkins et al. 2005, Zeisel, Freake et al. 2005) as well as the potential for the discovery of metabolites (biomarkers) that are indicative of disease (Ackermann, Hale et al. 2006, Kenny, Broadhurst et al. 2008), and the ability to monitor pharmacological therapies (Chen, Gonzalez et al. 2007) is very exciting.

Metabolomics involves the study of numerous analytes that have very diverse physical and chemical properties and occur in a wide concentration range. Currently there is no single instrument platform that can analyse all metabolites, and this may represent part of the reason that metabolomics lags behind genomics, transcriptomics and proteomics (Dettmer, Aronov et al. 2007). To further complicate the analysis of the metabolome, it must be taken into account that metabolite distributions are subject to high temporal and spatial variability e.g. circadian fluctuations in mammals (Dettmer, Aronov et al. 2007) and diet-dependent biological variability (Vigneau-Callahan, Shestopalov et al. 2001). Due to this complexity a number of different strategies are employed when considering the metabolome (Dunn 2008).

Tang et al (Tang, Wang 2006) have conjectured that the ideal detection method for metabolomic analysis would have to be objective, have high sensitivity and reproducibility, good signal resolution, not require pre-knowledge to assist biomarker discovery, have good quantification capability for

complex mixtures, be able to provide in depth molecular information such as structure, concentration, dynamics, interactions, pH and compartmentation, be holistic rather than selective, be inexpensive and have high throughput, require little or no sample preparation, be non-invasive and non-destructive to facilitate *in vivo* and *in situ* studies, and have low recurrent expenditure. In reality this analytical platform does not exist but the available techniques attempt to meet as many of these criteria as possible.

Most metabolomic analyses are carried out by mass spectrometry (MS), and nuclear magnetic resonance (NMR) spectroscopy (Fiehn, Kind 2007). MS requires pre-separation of the metabolic components, usually by either gas chromatography after chemical derivatisation, or liquid chromatography (Beckonert, Keun et al. 2007).

1.12.1 Mass Spectrometry

Mass Spectrometry is an established technique and is a potent platform for metabolomics due to its ability to detect metabolites at µM concentrations (Werner, Heilier et al. 2008). Atmospheric pressure ionisation (API) includes common soft ionisation techniques that generally produce protonated molecules $[M+H]^+$ or deprotonated molecules $[M–H]^-$. Some metabolites can only be observed in one ionisation mode, therefore acquisition should be performed in both positive and negative ion mode to maximise the metabolome coverage (Boccard, Veuthey et al. 2010).

The high mass accuracy of MS provides structural information, as an exact molecular mass can be indicative of the molecular formula or fragments of the molecular structure (Dunn, Ellis 2005).

Direct Injection MS (DIMS) is the simplest form of MS technology. It allows high throughput screening and is mainly used for sample classification. Quantification and metabolite identification is limited in this technique due its susceptibility to ion suppression, especially when performed on complex biological matrices (Boccard, Veuthey et al. 2010). An effective chromatographic separation prior to MS will reduce these effects (Cubbon, Antonio et al. 2010).

1.12.2 Gas Chromatography Mass Spectrometry

Combined Gas Chromatography Mass Spectrometry (GC-MS) is a system where volatile and thermally stable compounds are first separated by GC and then eluting compounds are detected traditionally by electron-impact mass spectrometers (Dunn, Ellis 2005). GC-MS provides reproducible and accurate measurements of volatile compounds and the fragmentation pattern of these molecules (Jonsson, Gullberg et al. 2004, Veriotti, Sacks 2001). The chemical derivatisation of semi-volatile compounds is required to increase the volatility and produces ions that can be separated in the GC column (Halket, Zaikin 2003, Halket, Waterman et al. 2005, Zaikin, Halket 2003). GC-MS has been called the “gold standard” in metabolomic analyses (Harrigan, Goodacre 2003) and libraries have been built to allow the identification of compounds (Schauer, Steinhauser et al. 2005).

Comprehensive two-dimensional gas chromatography (GC x GC) is a novel separation technique as conventional GC cannot resolve all analytes. Initially a heart-cut technique was used in which one or a limited number of fractions of the first column eluate was transferred to a second column with

different characteristics to obtain an improved separation (GC – GC). The technique was not useful for samples in which the analytes of interest are scattered throughout the first-dimension chromatogram, or in which attention has to be devoted to unknowns more than to target analytes. Therefore a comprehensive technique has evolved in which the entire sample is subjected to 2 independent separations. The 2 dimensional (2D) separation is completed in the run time of the first separation thus providing more information and reducing the time of GC – GC (Beens, Brinkman 2005). This technique is linked to MS in metabolomic analyses (GC x GC-MS).

1.12.3 Liquid Chromatography Mass Spectrometry

Liquid Chromatography Mass Spectrometry (LC-MS) is another combined technique in which metabolites are separated by liquid chromatography and then detected by electrospray ionisation (ESI) or less commonly API. This technique does not require samples to be volatile and is carried out at lower temperatures than GC-MS, thus simplifying sample preparation (Dunn, Ellis 2005). Although sample derivatisation is generally not required, it can be beneficial to improve chromatographic resolution and sensitivity or to provide ionisable groups on metabolites otherwise undetectable by ESI-MS (Leavens, Lane et al. 2002).

ESI does not result in fragmentation of molecular ions as observed in electron impact mass spectrometers, and therefore does not allow direct metabolite identification by comparison of ESI mass spectra (Dunn, Ellis 2005). However, with the use of accurate mass measurements and/or tandem MS (MS/MS) to provide collisional induced dissociation (CID) and related mass spectra (MS/MS), metabolite identification can be performed (Lenz, Bright et al. 2004).

The introduction of capillary LC (Dear, Ayrton et al. 1999, Ding, Sorensen et al. 2007) and ultra-high pressure LC (Swartz 2005) has great potential for metabolomic analyses on complex samples by improving the chromatographic performance.

1.12.4 Nuclear Magnetic Resonance Spectroscopy

Nuclear Magnetic Resonance (NMR) Spectroscopy is a rapid, quantitative, non-destructive, non-invasive, non-equilibrium perturbing technique with high throughput that requires minimal sample preparation. It provides detailed information on solution-state molecular structures, based on atom centred nuclear interactions and properties (Dunn, Ellis 2005, Beckonert, Keun et al. 2007). It is not however as sensitive as MS (Lenz, Wilson 2007).

NMR spectroscopy operates by the application of strong magnetic fields and radio frequency pulses to the nuclei of atoms. In atoms with an odd atomic number (e.g., ^{1}H) or odd mass number (e.g., ^{13}C), the presence of a magnetic field will cause the nucleus to possess spin, termed nuclear spin. Absorption of RF energy will promote the nuclei from low-energy to high-energy spin states, and the subsequent emission of radiation during the relaxation process is detected (Dunn, Ellis 2005).

It tends to be ^{1}H NMR spectroscopy that is employed in clinical studies (Dunn, Ellis 2005), which has been shown to be a robust and reproducible technique in metabolomic analyses (Dumas, Maibaum et al. 2006).

Table 1.7: Advantages and Limitations of Mass Spectrometry versus NMR in Metabolomic Analysis (Lin, Helsby et al. 2011)

	Mass Spectrometry	**NMR**
Quantitation & Reproducibility	Lower	Higher
Sensitivity	Higher	Lower
Detection Range	Wider • Couple with chromatographic separation (e.g. LC, GC)	Narrower • Bias towards high abundance metabolites • Restricted to specific resonances • Overlapping signals not easily resolved
Samples	Cannot be reused	May be nondestructive

1.12.5 Orbitrap

The Orbitrap, a mass spectrometer, operates by radially trapping ions about a spindle-like central electrode, kept at high voltage. An outer barrel-like electrode, kept at ground potential, is coaxial with the inner electrode and mass/charge values are measured from the frequency of the harmonic ion oscillations, along the axis of the electric field, undergone by orbitally trapped ions. The electrodes are shaped in such a way that the quadro-logarithmic potential distribution is formed with very high accuracy. Ion frequencies are measured non-destructively by acquisition of time-domain image current transients; with subsequent fast Fourier transforms (FFTs) being used to obtain mass spectra (Hu, Noll et al. 2005).

1.12.6 The Application and Reporting of the Metabolome in Biomarker Discovery

The “omics” technologies lend themselves to the possibility of biomarker discovery, especially using multiple biomarkers simultaneously to diagnose or predict disease. Statistically there are three steps in biomarker analysis; biomarker selection, performance evaluation, and model creation.

Biomarker selection involves the identification of an optimal subset of feature that will provide the maximal discriminating power between diseased and healthy samples. Performance evaluation involves the assessment and validation of the panel of biomarkers proposed. The final model creation involves developing a fixed mathematical model, which combines the panel of biomarkers into a single test score with the aim of accurately predicting a particular clinical outcome, given the measured biomarker responses from a particular target population.

Metabolomic studies can be placed into two general categories, those evaluating biological process, and those aiming to develop biomarkers. Studies aimed at biological understanding tend to utilise

multivariate statistical methods such as principal component analysis (PCA) or partial least squares discriminant analysis (PLS-DA). The results are accompanied by a long list of compounds selected based on a given model's loading values, variable projection scores, or p-values derived from parametric univariate hypothesis testing, or their non-parametric equivalent performed on each measure metabolite in turn. These compounds may be labeled "putative biomarkers", but may not be useful in clinical practice.

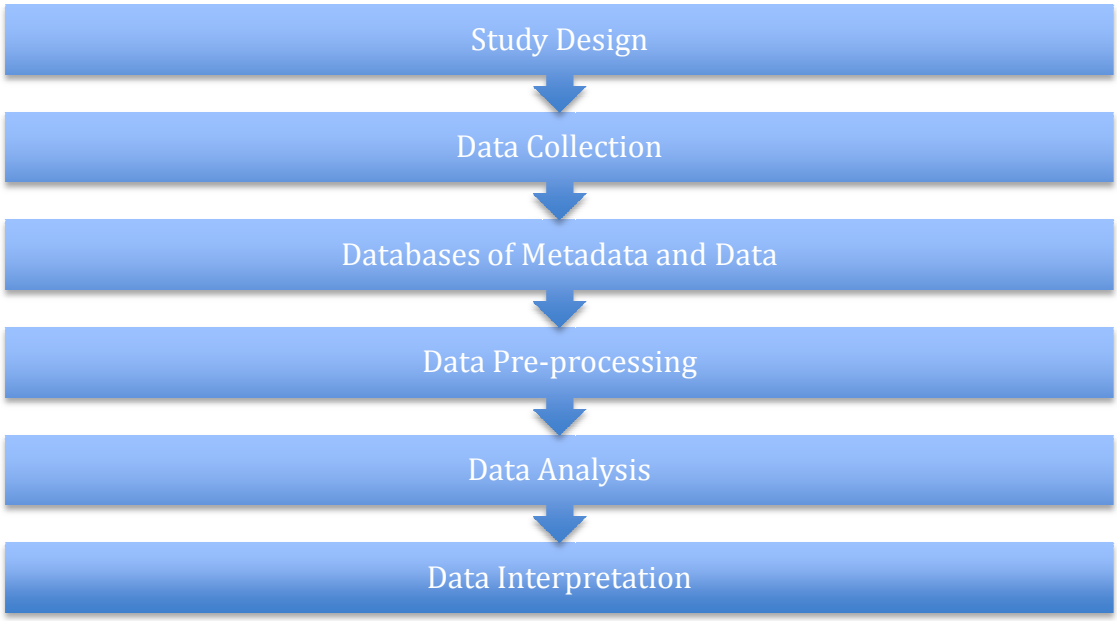
Studies focused on biomarker discovery should be performed *a priori* rather than *post hoc*. That is, biomarker selection must be performed before deriving a definitive multivariate predictive model. A short list of up to ten metabolites is mathematically more robust and more practical for clinical testing purposes. Supervised machine learning algorithms, or multivariate regression models should be used to build the predictive models for biomarker analysis.

Whilst metabolomic biomarkers screening for inborn errors of metabolism in neonates are in clinical practice, as they can often be diagnosed using a single metabolic marker (Scolamiero, Cozzolino et al. 2015), other chronic diseases exhibit smaller concentration changes spread across potentially dozens of metabolites, making the development of a single compound test very challenging. Compound quantification is another important area when considering metabolites as biomarkers, as it can be difficult and time-consuming (Xia, Broadhurst et al. 2013).

The standardisation of metabolomic profiling techniques, and the manner in which they are reported, is of utmost importance. There are many challenges starting with the vast number of samples types available for analysis, and the significant number of analytical platforms available, with MS and NMR being the most utilised. The complexity of the statistical processes required to analyse data, and the need for standardisation of both the experimental and reporting techniques, as well as validation of said techniques, lead to the publication of proposed minimum reporting stardards related to the chemical analysis aspects of metabolomics experiments including; sample preparation, experimental analysis, quality control, metabolite identification, and data pre-processing (Sumner, Amberg et al. 2007).

In metabolomics experiments, the numbers of samples is far fewer than the number of metabolites or variables measured. Statistical analysis (univariate or multivariate), or machine learning analysis, as well as informatic analyses, is therefore required to produce knowledge that can be useful in the generation of hypotheses and biological understanding. The Framework Programme 7 EU Initiative "COordination of Standards in MetabOlomicS" (COSMOS) is developing a robust data infrastructure and exchange standards for metabolomics data and metadata (Salek, Neumann et al. 2015). The development of metabolomics repositories, such as Metabolomics Workbench or MetaboLights, are an example of using standardised legacy data to demonstrate new, or compare existing data analysis approaches, where data standards and sharing excel. These repositories also facilitate computational advances that would not previously have been possible (Rocca-Serra, Salek et al. 2016).

Figure 1.7: Information flow in a metabolomics experiment



1.12.6.1 Data Reduction and Deconvolution

The data produced from metabolomic profiling produces multiple measurements of multiple metabolites at different concentrations. These large volumes of raw data need to be transformed into an appropriate data format that can be statistically analysed. This involves the extraction of relevant signals from the data, and correcting for confounding factors that may bias the data, including biofluid characteristics and analytical processing techniques. In metabolomic terms, deconvolution, meaning the separation of overlapping signals into individual chemical peaks, is used in hyphenated chromatography / mass spectrometry, where the raw 3D matrices (time vs. mass vs. intensity) are especially complex. In NMR, deconvolution describes the separation of overlapping peaks into individual resonances or into metabolite identification lists, and the term “data transformation” is preferred (Goodacre, Broadhurst et al. 2007).

1.12.6.2 Pre-processing and Pre-treatment

Pre-processing is a generic term for methods used to convert raw instrumental data to clean data to allow data processing. Pre-treatment involves transforming the clean data to make them ready for data processing.

There are many methods for pre-processing data matrices produced by metabolomic studies, and these, including the order in which they were performed, and the software utilised, should be reported. Order-independent pre-processing methods are more difficult to report as they may require assessment or interpretation by the reader prior to the analysis moving on the the next stage, such as outlier detection by principal component analysis (Goodacre, Broadhurst et al. 2007).

1.12.6.3 Univariate Analysis

Metabolomics experiments produce multivariate data, however, univariate methods can be utilised to test individually for metabolites that are increased or decreased significantly between different groups. These tests include parametric methods for data that are normally distributed, such as ANOVA (analysis of variance), *t*-tests, and *z*-tests. When the data is not normally distributed, or cannot be assumed to be so, non-parametric methods can be used such as the Kruskal-Wallis test (Goodacre, Broadhurst et al. 2007).

1.12.6.4 Multivariate Analysis

The volume of "outputs" created from a metabolomics experiment is vast, thousands from a few subjects. The initial data analysis starts from a single matrix N x D, where N is the number of samples, and D is the number of variables (metabolomics / masses / retention times etc.). Each independent variable may be thought of as a single geometric dimension, such that each sample may be considered to exist as a single point in an abstract entity referred to as D-dimensional hyperspace. In order to determine the underlying properties of the data points in this hyperspace, methods such as unsupervised dimensionality reduction (usually projection into a space of significantly smaller dimensionality whilst trying to minimise information loss), clustering (identifying groups of points that are more similar to each other than the rest of the data), correlation analysis, or supervised pattern recognition / machine learning (including multivariate regression and discriminant analysis) may be employed (Goodacre, Broadhurst et al. 2007).

1.12.6.5 Unsupervised Methods

Unsupervised methods can be split into dimension reduction and cluster analysis. Dimension reduction, typified by principal component analysis (PCA), is essentially a multivariate linear transformation, and is often used as a pre-processing step prior to application of a supervised method such as partial least squares and discriminate analysis (PLS-DA). When reporting this method, reference to the software used or the theoretical source of the mathematical algorithm should be made, as well as stating any assumptions made about the characteristics of the dataset before transformation.
Cluster analysis utilises an algorithm attempting to "find" clusters of similarly characterised samples. Once found they may be used to classify each sample and similarities between clusters may be assessed. When reporting this method, details of the software or algorithm must be given, as well as the similarity measure used. It is also possible to determine correlations between the metabolites or variables by conducting Correlation Analysis, and creating correlation matrix pseudomaps or confusion matrices as the first step in data exploration (Goodacre, Broadhurst et al. 2007).

1.12.6.6 Supervised Methods

When one knows the classes or value of the responses that one is trying to predict (*Y*-data) associated with each of the sample inputs (*X*-data), supervised methods can be used. The aim is to find a model that will correctly associate all of the *X*-data with the target *Y*-data. Usually supervised methods

require meta-parameterisation (meta-parameters are parameters that help to define the structure and optimisation of the model) (Goodacre, Broadhurst et al. 2007).

1.12.6.7 Model Validation

Should the results of a metabolomics study indicate a general model, or general biomarker discovery, reports must be verified by a model validation. This is required in both supervised and unsupervised analysis. Internal validation is used in supervised methods to optimise meta-parameters such as the number of latent variables in PLS. Even if internal validation appears to improve the predictive capability of the final model chosen, this model's generalisation capability tends to be overoptimistic (overfitting); hence it is not a replacement for extrinsic model validation.

Simple model validation involves splitting the data into three sets: training, monitoring, and test. The training data are used to build one or more possible models, the monitoring data are then used to assess and optimise the quality of every "trained" model, and the independent test data are used to measure the generalisation / predictive expectation of the final published "optimal" model. There are several validation methods in which the training and monitoring sets are initially combined and then subsequently dynamically partitioned into temporary training / monitoring dataset (e.g. bootstrapping and K-fold cross-validation).

Importantly, in all cases, the test set is "blind" to the model building and selection process. The test procedure involves applying the test data to the "optimal" model with subsequent model predictions being compared to known responses. These models are known to be overoptimistic.

For unsupervised methods sush as PCA, the monitoring dataset is not required. However, care must be taken not to make claims regarding data clustering when plotting the principal component y against principal component x when x and y are anything other than 1 and 2 respectively. Searching all possible component axes is a form of multiple testing (supervised analysis) and therefore requires the application of proper corrections to the clustering statistic (e.g. Bonferroni) (Goodacre, Broadhurst et al. 2007).

1.13 Metabolomic Studies

1.13.1 Human Diseases

Metabolomic studies have been carried out in numerous human diseases in an aim to aid both diagnosis and disease monitoring, as well as personalising treatments. Many metabolomic studies into malignancies (e.g. liver (Wei, Suryani et al. 2012, Gao, Lu et al. 2009, Yang, Xu et al. 2004), bladder (Issaq, Nativ et al. 2008), prostate (Serkova, Gamito et al. 2008, Sreekumar, Poisson et al. 2009), oral (Zhou, Xu et al. 2009, Tiziani, Lopes et al. 2009, Yan, Wei et al. 2008), stomach (Hirayama, Kami et al. 2009), renal (Kim, Aronov et al. 2009, Kind, Tolstikov et al. 2007), brain (Chen, Lou et al. 2011, Monleon, Morales et al. 2008), lung (Chen, Ma et al. 2015), colorectal (Hirayama, Kami et al. 2009, Monleon, Morales et al. 2009, Mal, Koh et al. 2009, Denkert, Budczies et al. 2008, Chan, Koh et al. 2009) and breast / gynaecological cancers (Denkert, Budczies et al. 2006, Woo, Kim et al. 2009, Frickenschmidt, Frohlich et al. 2008)) have been undertaken in a search for biomarkers of disease and

disease activity. None, however, are currently in clinical use. Metabolomic profiling has also been undertaken in diabetes (Sas, Karnovsky et al. 2015), cardiovascular diseases (Basak, Varshney et al. 2015, Kordalewska, Markuszewski 2015) and neurological diseases (Zhang, Sun et al. 2013, Hassan-Smith, Wallace et al. 2012) with the aim of gaining knowledge of disease pathogenesis as well as searching for biomarkers. As yet this has not been translated into clinical practice either.

1.13.2 Animal Models of IBD

Varma et al (Varma, Bird et al. 2007) studied 30 male Sprague-Dawley rats. These were divided into three groups of ten rats each. The first group was fed a regular diet for 2 weeks, the second group was fed a regular diet for one week and 2% carrageenan by weight for the second week, and the third group was fed 2% carrageenan by weight for the two week duration. Degraded lambda-carrageenan has been shown to cause inflammation similar to human IBD in a rodent model (Moyana, Xiang et al. 1994).

After the two week period all rats were sacrificed, their colons were excised and the mucosal layer scraped off using a glass slide. ^{1}H NMR Spectroscopy experiments were carried out on the samples.

After correlating the spectroscopy results with histology results and performing a multivariate analysis, the accuracy with which these samples were assigned to their respective groups was 82%. Four regions of the spectra were identified as being the most discriminatory. These included spectral resonances due to the -CH_2HC= group in fatty acyl chain of triglycerides (2.79–2.83 ppm), creatine (3.00 ppm), phosphocholine (3.56–3.60 ppm) and glycerol backbone of lipids (4.03–4.05 ppm).

Martin et al (Martin, Wang et al. 2007) have characterised the metabolic profile of normal rodent intestinal tissue including proximal and distal colon using high resolution ^{1}H NMR Spectroscopy. They observed significant levels of phosphatidylcholine, creatine, glycerol and triglycerides. Phosphatidylcholine is the predominant phospholipid present in the cell membranes (Cullis, Hope 1992) including colonic mucosal cells (White 1973), and is an important constituent of PAF. PAF has been shown to increase significantly in mucosal biopsies from ulcerative colitis patients (Thyssen, Turk et al. 1996), and PAF levels in stool can be used to estimate the severity of mucosal inflammation in patients with IBD (Hocke, Richter et al. 1999).

Creatine is a metabolite present in normal tissues and serves as an energy reservoir. Colonic inflammation causes cellular damage and increases energy requirements therefore creatine concentrations may be increased during periods of inflammation.

Varma et al (Varma, Bird et al. 2007) concluded that ^{1}H NMR Spectroscopy is a sensitive tool to detect early colonic inflammation in an animal model of IBD.

Murdoch et al (Murdoch, Fu et al. 2008) studied urinary metabolomic profiles in IL-10 gene deficient (IL-$10^{-/-}$) mice, and compared them to wild-type mice using NMR Spectroscopy. IL-$10^{-/-}$ mice are genetically susceptible to develop enterocolitis when colonised with enteric mouse microbiota (Kuhn, Lohler et al. 1993). The urinary metabolic fingerprint of IL-$10^{-/-}$ and wild-type mice was found to be similar at 4 weeks, before development of IBD in the IL-$10^{-/-}$ mouse model. Those metabolites that showed significant differences when data were analysed by two-way analysis of variance (ANOVA)

(with $p < 0.01$) with onset of IBD at approximately 12 weeks and older were fucose ($p < 0.0001$); trimethylamine ($p < 0.0001$); dimethylamine ($p < 0.0001$); fumarate ($p < 0.0001$); *N*-isovaleroylglycine ($p < 0.0001$); 2-oxoglutarate ($p = 0.0008$); trimethylamine-*N*-oxide ($p = 0.0011$); butyrate ($p = 0.0025$); citrate ($p = 0.0027$); succinate ($p = 0.0035$); 3-indoxylsulfate ($p = 0.0036$); valine ($p = 0.0058$); and phenylacetylglycine ($p = 0.0087$).

This study was the first to demonstrate urinary metabolic differences in an IBD mouse model as the disease develops over time through serial metabolic profiling. It also demonstrates significant differences between the urinary metabolites of wild-type mice and IL-$10^{-/-}$ mice, however the significance if these findings is yet to be clarified. Potentially these findings could lead to a non-invasive urinary biomarker of IBD.

Lin et al (Lin, Edmunds et al. 2009, Lin, Barnett et al. 2010) also performed urinary metabolomic analyses on IL-$10^{-/-}$ mice, however the GC-MS platform was used in these studies.

Elevated urinary levels of xanthurenic acid and fucose were identified indicating upregulation of tryptophan catabolism and perturbed fucosylation in IL-$10^{-/-}$ mice. Three short-chain dicarboxylic acid metabolites (glutaric acid, 2-hydroxyglutaric acid, and 2-hydroxyadipic acid) were decreased in IL-$10^{-/-}$ mice relative to wildtype, suggesting the downregulation of fatty acid oxidation (Lin, Edmunds et al. 2009).

Martin et al (Martin, Rezzi et al. 2009) studied 20 wild-type mice and 20 IL-$10^{-/-}$ mice using a combination of histopathological analysis of intestinal sections, metabolic profiling of blood plasma using ^{1}H NMR Spectroscopy, and measurement of plasma inflammatory biomarkers.

Histology of the colon and caecum showed inflammatory changes in all IL-$10^{-/-}$ mice at the age of 8, 16 and 24 weeks, the grade of which increased with age. Concentrations of serum amyloid A (SAA) and soluble tumour necrosis factor II (sTNFRII) were measured. Expression of SAA showed an upward trend in IL-$10^{-/-}$ mice compared to the wild-type, however the difference never reached significance. Blood levels of sTNFRII were significantly increased in IL-$10^{-/-}$ mice from the age of 8 weeks onwards.

On metabolic analysis, IL-$10^{-/-}$ mice showed a marked reduction of global levels of creatinine, dimethylglycine, glucose, leucine, methionine, trimethylamine, tyrosine and very low density lipoprotein (VLDL). These changes were associated with an increase in alanine, arginine, choline in phospholipids, citrate, fumarate, high density lipoprotein (HDL), isoleucine, lactate, low density lipoprotein (LDL), phenylalanine, polyunsaturated lipids and pyruvate.

The relative decrease of the level of VLDL particles in IL-$10^{-/-}$ mice can be associated with an increased activity of lipoprotein lipase in the capillary beds in adipose tissue and skeletal muscles to remove triglycerides.

Inflammatory processes in IL-$10^{-/-}$ mice were characterised by increased blood levels of secretory phospholipase A2, which promotes hydrolysis of phospholipids in LDL and VLDL and generates PUFAs that can be oxidised and promote inflammation.

Elevated levels of lactate, citrate and pyruvate and decreased plasma glucose level in the IL-$10^{-/-}$ mice are suggestive of increased fatty acid oxidation and extensive glycolysis to accommodate a higher

energy demand or decreased energy availability due to decreased nutrient absorption through the compromised alimentary system.

Elevation of alanine and glutamine in plasma was associated with decreased levels of the branched-chain amino acids valine and leucine, which reflected both breakdown of proteins and increased gluconeogenesis and carbon flux through the anapleurotic pathway to support ATP production.

Schicho et al (Schicho, Nazyrova et al. 2010) used ^{1}H NMR Spectroscopy to perform quantitative metabolomic analysis of metabolites in urine and blood serum of mice in which experimental ulcerative colitis has been induced with dextran sulfate sodium (DSS). DSS induced colitis is one of the most widely used and reproducible animal models for UC (Okayasu, Hatakeyama et al. 1990).

During DSS colitis in urine samples increases in concentrations were observed for the amino acids creatine, carnitine, glycine and phenylalanine, for allantoin and for methylamines such as dimethyamine, trimethylamine and trimethylamine-*N*-oxide while urea (a terminal metabolite of catabolic processes), methionine, nicotinamide and ascorbate were decreased.

In serum, large increases in concentration are shown for ketone bodies (acetoacetate, acetone and 3-hydroxybutyrate), hypoxanthine, inosine and tryptophan, while glucose, Krebs cycle intermediates (fumarate, 2-oxoglutarate, citrate), and several amino acids are decreased.

Methylamines are produced by gut bacteria (Smith, Wishnok et al. 1994), which are known to have a role in IBD pathogenesis (Takaishi, Matsuki et al. 2008).

Overall, urine samples revealed mostly changes in bacterial metabolites and metabolites associated with oxidative stress, while serum samples showed changes in metabolites associated with the regulation of the organism's energy level.

Chen et al also studied (Chen, Shah et al. 2008) DSS induced colitic mice, however they used LC-MS to perform metabolomic analysis of serum. They showed that DSS-treated mice have increased serum levels of stearoyl lysophosphatidiylcholine and decreased levels of oleoyl lysophosphatidiylcholine. The alterations in the levels of these lipids led to the discovery that these levels were caused by DSS inhibition of stearoyl-coA desaturase 1 (SCD1)-mediated olecic acid biogenesis in the liver, resulting in exacerbation of proinflammatory responses. The findings of the study implied that SCD1 and associated lipids could be potential drug targets for treating IBD (Lin, Helsby et al. 2011).

These animal models help to understand more about the pathogenesis of IBD but do not provide complete answers at present.

1.13.3 Human IBD Studies

Marchesi et al (Marchesi J.R., Holmes E. et al. 2007) used ^{1}H NMR Spectroscopy to study faecal extracts obtained from patients with CD and UC, as well as healthy controls. In the CD and UC patients levels of short chain fatty acids (SCFAs) butyrate, acetate, methylamine, and trimethylamine were reduced when compared to the control population. The changes were more marked in the CD group than the UC group.

Several studies (Seksik, Rigottier-Gois et al. 2003, Sokol, Seksik et al. 2006, Scanlan, Shanahan et al. 2006) of the diversity of microbiota associated with IBD have shown that members of the *Clostridium*

coccoides and *Clostridium leptum* groups are significantly reduced when compared to healthy subjects. These bacterial groups are involved in SCFA production, and therefore these findings would be consistent with Marchesi's results.

Jansson et al (Jansson, Willing et al. 2009) used ion cyclotron resonance-Fourier transform mass spectrometry (ICR-FT/MS) to study the metabolites in 17 identical twin pairs including healthy twin pairs, concordant pairs (both twins have CD) and discordant pairs (one twin is healthy and the matched twin has CD). ICR-FT/MS with an ultrahigh mass resolution enables differentiation of very subtle variations in thousands of mass signals, including higher molecular weight metabolites (Rossello-Mora, Lucio et al. 2008).

Siffledeen et al (Siffledeen, Fu et al. 2008) were the first to use NMR Spectroscopy to observe differences in the urinary metabolomic profile between patients with IBD and healthy controls (78% sensitivity and 89% specificity on a blinded test set). They also found that patients with CD could be differentiated from those with UC.

Williams (Williams, Cox et al. 2009) analysed samples of urine from patients with CD, UC and healthy controls. ^{1}H NMR Spectroscopy was used to carry our urinary metabolomic profiling. Hippurate levels were found to be significantly lower in CD patients than in the other groups ($p<0.0001$) as were 4-cresol sulphate levels ($p<0.0002$). Formate levels were found to be significantly higher in CD patients than in UC patients or healthy controls ($p<0.0005$).

Urinary hippurate is a product of the microbial metabolism of various dietary aromatic compounds (e.g. purines) and aromatic amino acids (e.g. tyrosine) to benzoic acid with subsequent renal and hepatic conjugation of benzoic acid with glycine (Williams, Cox et al. 2009). The concentration of urinary hippurate has been shown to be modulated according to gut microbes (Nicholls, Mortishire-Smith et al. 2003, Williams, Eyton-Jones et al. 2002). A reduction in *Clostridia* spp. has been shown in IBD (Sartor 2008), and Li et al (Li, Wang et al. 2008) found a positive association between *Clostridia* spp and hippurate levels. This leads us to believe that there is an inherent difference in the gut microbiotia between patients with IBD and healthy controls.

Mammalian formate may be generated endogenously as the result of one-carbon metabolism (Case, Benevenga 1977), however it is also a major fermentative microbial metabolite (Leonhartsberger, Korsa et al. 2002). It is a characteristic product in the mixed-acid fermentation of the *Enterobacteriaceae*. The result of increased formate in the CD group is consistent with previous findings of an increase in *Enterobacteriacea* and particularly in *E. coli*, in CD patients (Seksik, Rigottier-Gois et al. 2003, Sartor 2008).

Urinary 4-cresol sulphate is the product of the bacterial metabolism of tyrosine (Folin, Denis 1915) and is only synthesized by few bacterial species, particularly by *Clostridia* spp. and, to a lesser extent, by *Bacteroidetes* (Bone, Tamm et al. 1976, Smith, Macfarlane 1996). *Clostridia* spp. As previously discussed *Clostridia* spp. has been shown to be reduced in CD, and to a certain extent the same has been suggested about *Bacteroidetes* (Ott, Musfeldt et al. 2004, Baumgart, Dogan et al. 2007). This would be in keeping with the findings of reduced 4-cresol sulphate in the urine of CD patients in this study.

Balasubramanian et al (Balasubramanian, Kumar et al. 2009) recruited patients with CD and UC, as well as healthy controls to study the metabolism of the colonic mucosa using ^{1}H NMR Spectroscopy. In the active phase of UC and CD, significantly lower ($p \leq 0.05$) concentrations of amino acids (isoleucine, leucine, valine, alanine, glutamate and glutamine), membrane components (choline, glycerophosphorylcholine and myo-inositol), lactate and succinate were observed compared to normal mucosa of controls. An increased level of α-glucose was found in the colonic mucosa of active IBD patients when compared to the controls. These findings essentially translate into lower carbohydrate and protein metabolism in the active phase of IBD leading to loss of mucosal integrity.

In normal intestinal mucosa glucose serves as an energy source (Roediger 1982, Ardawi, Newsholme 1985). Its utilisation and, consequently, lactate production are reduced during malnutrition and starvation, a common symptom observed in IBD (Firmansyah, Penn et al. 1989, Faintuch 2002, Al-Jaouni, Hebuterne et al. 2000). Low levels of lactate and high levels of α-glucose observed in this study indicate the inability of the colonic mucosal cells to utilise glucose for its energy needs in UC and CD. In the remission phase of UC and CD, the concentration of most of the metabolites was similar to the controls except for lower values of lactate, glycerophosphorylcholine and myo-inositol in UC and lactate in CD. Formate levels were found to be significantly lower in patients with the active phase of UC compared to patients with the active phase of CD. This raises the potential to use formate as a biomarker in distinguishing between CD and UC in the active phase.

Bjerrum et al (Bjerrum, Nielsen et al. 2010) studied mucosal colonic biopsies, colonocytes, lymphocytes, and urine of patients with active UC, quiescent UC and healthy controls. Metabolomic analyses were performed using ^{1}H NMR Spectroscopy. In the biopsies from patients with active UC higher levels of antioxidants, ascorbate and glutathione, and of amino acids, glutamate, glutamine, taurine and asparate, but lower levels of lipid, glycerophosphocholine, *myo*-inositol, and betaine were found, whereas the colonocytes only displayed low levels of glycerophosphocholine, *myo*-inositol and choline. The increased levels of glutamine and glutathione found in the biopsies from patients with active UC are most likely due to activated lymphocytes in the lamina propria as these metabolites were also found to be prominent features of the metabolic profile of peripheral lymphocytes. The results of this study lead to a hypothesis of an imbalanced antioxidant response in the lamina propria and colonocytes of patients with UC.

Unfortunately at present there are inconsistencies within the literature regarding amino acid profiles and their part in the pathogenesis of IBD. Glutamine and glutathione have been studied previously however Balasubramanian et al (Balasubramanian, Kumar et al. 2009) in fact found levels to be lower than normal, rather than raised as found in this study. Finnie et al (Finnie, Taylor et al. 1993) observed increased glutamine metabolism in the distal colon of patients with quiescent UC, whereas Chapman et al (Chapman, Grahn et al. 1994) and Duffy et al (Duffy, Regan et al. 1998) found no such difference. Similar inconsistencies have been found with respect to glutathione (Ruan, Rao et al. 1997, Holmes, Yong et al. 1998). This is potentially due to the heterogeneity of these studies.

The metabolic profiles of lymphocytes and urine did not allow a differentiation between active UC, inactive UC, and controls, which means that the inflammation in the large bowel is not reflected systemically in a cohort with moderate UC.

Bezabeh et al (Bezabeh, Somorjai et al. 2001) used ^{1}H NMR Spectroscopy to analyse colonic biopsies from IBD patients and normal healthy controls. The aim was to determine whether it was possible to differentiate between CD, UC and healthy controls. A classification accuracy between UC and CD of 98.6% was achieved, with only one case of CD and no cases of UC misclassified. The classification accuracy between normal controls and IBD was 97.9%. They felt that there is strong potential for NMR Spectroscopy to be used in the accurate diagnosis of indeterminate colitis; however this method does require colonic biopsies.

Johnston et al (Johnston 2014) were the first group to use an UPLC-MS platform to study the metabolomic profiles of serum from patients with CD and UC, and compare them to healthy controls. In their study, the majority of metabolic differences were observed in Vitamin D and its metabolites, steroids and their derivatives, fatty acids, bile acids, phospholipids and phosphocholine. A number of metabolites were uniquely altered in one or other IBD subclass versus control comparisons. Sphingolipids were exclusively decreased within the CD cohort compared to the control group. Isoprenoids were decreased in the UC cohort compared to controls. A comparison of UC and CD profiles showed that the quinone, ubiquinone was significantly greater in the UC group. All of these findings were of statistical significance at the discovery stage and replicated through a validation study.

1.13.4 Metabolomics and Personalised Medicine in IBD

Metabolomic profiling has the potential to be used to determine responders from non-responders when considering drug therapies. In IBD, immunosuppressant drugs such as thiopurines (azathioprine or mercaptopurine) are used to induce and maintain long-term remission. However there is the risk of significant adverse effects such as myelosupression with these drugs, which can occur through the accumulation of mercaptopurine derived thioguanine nucleotide (TGN) cytotoxic metabolites that are linked to a lack of red blood cell thiopurine S-methyltransferase (TPMT) activity. Prior to commencing treatment with thiopurines, TPMT activity is measured to identify the 1:300 patients who are at risk of severe myelosuppression. Despite monitoring, myelotoxicity can still occur with normal TPMT activity, indicating a role for TPMT in the prediction of early events rather than in long-term control (Lennard 2002).

2

Hypothesis and Principle Research Objectives

2.1 Hypothesis

Currently there is little in the literature studying metabolomic profiles of patients with IBD. As previously discussed, Johnston et al (Johnston 2014) studied both serum and urinary metabolomic profiles in both CD and UC and compared them to healthy controls. During discovery and validation studies important metabolomic differences were defined between CD, UC and matched controls. Univariate analysis demonstrated greater than 50 metabolites that varied in their relative concentration when comparing CD or UC versus control patients, whilst only 6 metabolites were shown to change when comparing CD versus UC. The greatest perturbation to metabolism was between healthy and disease subjects as would be expected, with a significantly lower level of differences observed between the two diseases. This may highlight similar pathophysiological mechanisms of both diseases with spatial differences in symptoms.

Using metabolomic profiles measured on GC-ToF-MS and UHPLC-FTMS platforms to analyse serum and urine samples from healthy controls, CD and UC patients we hypothesise that:

1. The work of Johnston et al (Johnston 2014) can be replicated with regards to metabolite identification and differentiation between healthy controls and IBD
2. Metabolomic profile will alter with disease activity, anatomical location and treatment

2.2 Principle Research Objectives

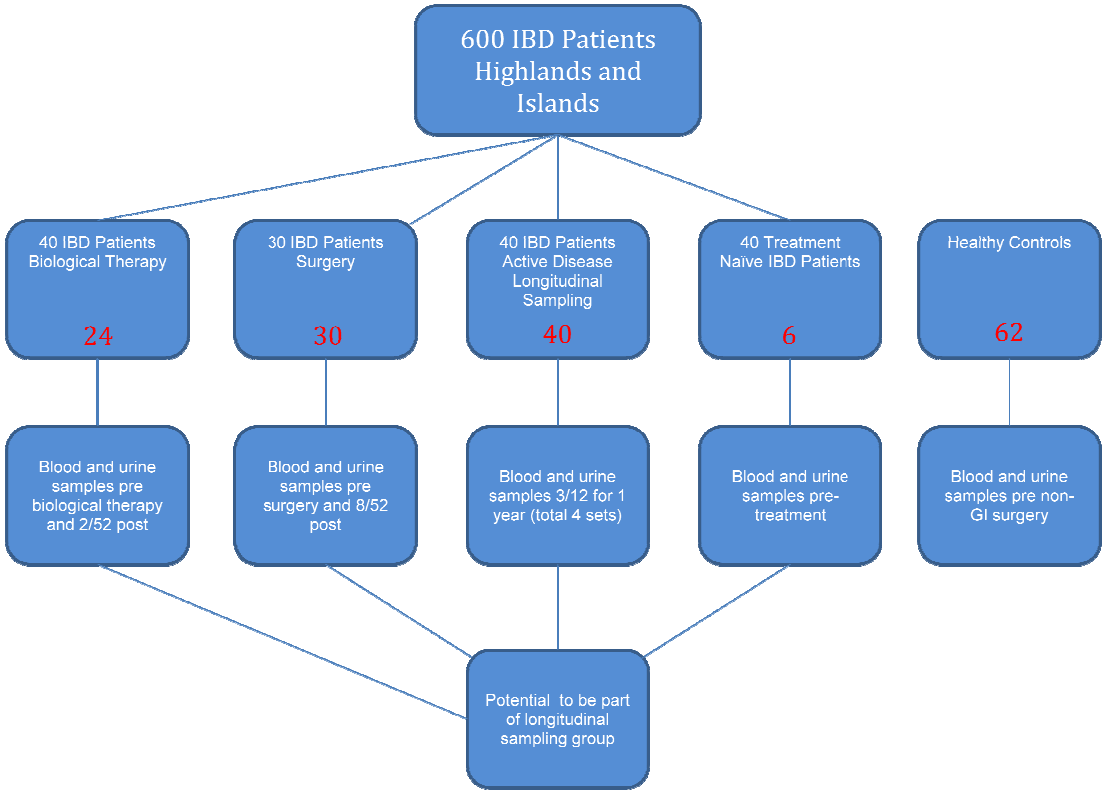
1. To determine whether it is possible to differentiate between the metabolmic profiles of healthy controls, ulcerative colitis patients and Crohn's disease patients.
2. The identification of biologically relevant metabolites in IBD.
3. To determine whether metablomic profile in IBD is affected by disease activity, anatomical location, age of onset or treatment.

3

Materials and Methods

3.1 Patients, Materials and Methods

Figure 3.1: Study Design



The design of the study, in association with Professor Roy Goodacre and Dr Warwick Dunn, was based around the IBD cohort being treated in NHS Highland (Appendix 8.1). Each year approximately 40 new patients are diagnosed with IBD, 40 patients commenced biological therapy and 30 underwent surgery for IBD. These were the figures decided upon for attempted recruitment, as shown in Figure 3.1 in white. The number of patients successfully recruited into each group is shown in Figure 3.1 in red. This correlates with the recommendations made by Lancaster et al for the number of participants required for a pilot study (Lancaster, Dodd et al. 2004).

Throughout the study it was feasible for patients to cross from the longitudinal sampling group into either or both of the biological and surgical groups, depending on the treatment that they required. It was also possible for patients initially recruited into the surgical or biological groups to join the longitudinal sampling group, unless they had potentially curative surgery.

The treatment naïve group of IBD patients was a cohort we felt may give the "purest" metabolomic profile as we hoped not to see drug contamination or alteration of the profile in response to treatment. This was a challenging group to recruit, resulting in low numbers, however due to the percieved value of this uncontaminated profile we chose to continue with analysis.

The biofluids of urine and serum were utilised in view of the relatively non-invasive and simple sampling methodology.

3.2 Ethical Approval

Ethical approval was granted on 17/06/11 by the North of Scotland Research Ethics Committee; Reference Number 11\AL\0238. The approved documentation is attached in Appendix 8.1.1.

3.3 Patient Recruitment

Patients with IBD in the Highlands and Islands Scotland were approached to take part in the study by means of a letter of invitation with a routine clinic or endoscopy appointment, or were approached by the clinician in charge of their care in either the in- or out-patient setting.

Each participant in the study was recruited by me. Those willing to discuss potential recruitment were given information sheets detailing the project, and the opportunity to ask questions regarding the project of myself. Exclusion criteria were those under the age of 16 years, and those unable to give informed consent. Those willing to participate in the study gave written informed consent to me (Appendix 8.1.2-8.1.14). A detailed interview and review of the case notes was carried out by me to establish relevant demographic and clinical data. This included classification of diet, although a formal food diary was not undertaken. A review of clinical data as well as disease activity scoring was carried out for each patient every time samples were collected. In view of their simplicity and use in routine clinical practice, the Harvey Bradshaw Index (Harvey, Bradshaw 1980) was used in patients with CD, and the Simple Clinical Colitis Activity Index (Walmsley, Ayres et al. 1998) was used in patients with UC. Disease phenotyping was carried out using relevant clinical investigations and pathological results available, by means of the Montreal Classification (Satsangi, Silverberg et al. 2006) in CD, and in UC, disease location / extent was recorded using the Paris Classification (Levine, Griffiths et al. 2011).

3.4 Harvey-Bradshaw Index

In 1980, Harvey and Bradshaw developed and validated a simplified index of CD activity based on 5 items; general well being, abdominal pain, number of liquid stools per day, the presence of an abdominal mass and the presence of complications (Harvey, Bradshaw 1980). They compared the simplified index (the Harvey-Bradshaw Index (HBI)) with the CDAI in a prospective study of 112 patients and found that there was significant correlation between the 2 despite not weighting the simple index and not averaging symptoms over one week. They did however state that symptoms and stool frequency should be scored from the day previous as a visit to the clinic may alter symptoms.

This simple index is reproducible and easy to use in a clinical setting; however it is reasonably subjective and is heavily biased by the number of liquid stools per day.

Table 3.1: Harvey-Bradshaw Index (Harvey, Bradshaw 1980)

General well-being
0 = very well
1 = slightly below par
2 = poor
3 = very poor
4 = terrible
Abdominal pain
0 = none
1 = mild
2 = moderate
3 = severe
Number of liquid stools per day
Abdominal mass
0 = none
1 = dubious
2 = definite
3 = definite and tender
Complications: arthralgia, uveitis, erythema nodosum, aphthous ulcers, pyoderma gangrenosum, anal fissure, new fistula, abscess (score 1 per item)

3.5 Simple Clinical Colitis Activity Index

In 1998, Walmsley et al (Walmsley, Ayres et al. 1998) described a simple clinical index to aid in the evaluation of UC exacerbations. They aimed for an index that could be calculated in an outpatient setting and that did not require physical examination, endoscopic examination or laboratory test results. The Simple Clinical Colitis Activity Index (SCCAI) was established by correlating criteria in the Powell-Tuck Index as well as nocturnal stool frequency, urgency of defaecation and the general well being factor from the Harvey-Bradshaw Index. On testing this score showed a highly significant correlation with both the Powell-Tuck score ($p<0.0001$) as well as all laboratory markers ($p=0.0003$ to $p<0.0001$) (Walmsley, Ayres et al. 1998).

Clinical remission or response to medication did not have scores allocated in the original paper, however a score of <2.5 points has been shown to correlate with Patient-Defined Remission (Higgins, Schwartz et al. 2005). This score has not been formally validated (Cooney, Warren et al. 2007).

Table 3.2: Simple Clinical Colitis Activity Index (Walmsley, Ayres et al. 1998)

Symptom	Score
Bowel frequency (day)	
1 – 3	0
4 – 6	1
7 – 9	2
>9	3
Bowel frequency (night)	
1 – 3	1
4 – 6	2
Urgency of defecation	
Hurry	1
Immediate	2
Incontinence	3
Blood in stool	
Trace	1
Occasionally frank	2
Usually frank	3
General well being	
Very well	0
Slightly below par	1
Poor	2
Very poor	3
Terrible	4
Extracolonic manifestations	1 per item

3.6 The Montreal Classification

The Montreal Classification of CD accounts for age at onset of disease, disease location and disease behaviour to be taken into consideration in both clinical practice as well as for research purposes (Satsangi, Silverberg et al. 2006).

Table 3.3: The Montreal Classification (Satsangi, Silverberg et al. 2006)

Age at Diagnosis	• A1 below 16 years • A2 between 17 and 40 years • A3 above 40 years
Location	• L1 ileal • L2 colonic • L3 ileocolonic • L4 isolated upper disease *
Behaviour	• B1 non-stricturing, non-penetrating • B2 stricturing • B3 penetrating • p perianal disease modifier ‡
* L4 is a modifier that can be added to L1 – L3 when concomitant upper gastrointestinal disease is present. ‡ "p" is added to B1 – B3 when concomitant perianal disease is present.	

3.7 The Paris Classification

The Paris Classification of UC records the extent of colonic involvement. In paediatrics this classification also accounts for whether or not the patient has ever had a severe episode (as defined by the Paediatric Ulcerative Colitis Activity Index score ≥ 65) (Levine, Griffiths et al. 2011). This part of the score was not utilised in our adult population.

Table 3.4: The Paris Classification (Levine, Griffiths et al. 2011)

Extent	• E1: ulcerative proctitis • E2: Left-sided UC (distal to splenic flexure) • E2+: Left-sided UC (extending beyond the limitation of the endoscopic examination) • E3: Extensive (hepatic flexure distally) • E4: Pancolitis (proximal to hepatic flexure)
Severity	• S0: never severe • S1: ever severe

3.8 Healthy Control Recruitment

Healthy controls attending clinics for non-gastrointestinal operative procedures were approached initially by the clinician in charge of their care and then by a member of the research team if they were willing.

They were given information sheets detailing the project and the opportunity to ask any questions. Informed consent was gained and a detailed interview and review of the case notes was carried out to establish relevant demographic and clinical data. As previously exclusion criteria were under the age of 16 years, and unable to give informed consent.

3.9 Metadata

All relevant clinical data for the IBD patients and the healthy controls was stored on a secure Microsoft Access 2003 database on the NHS server. This database contained details of age, sex, body mass index, ethnicity, disease activity score (HBI / SCCAI), disease classification (Montreal / Paris), past medical and surgical history, current medications and family history of immunological diseases.

3.10 Sample Collection

Blood samples (up to 20ml) were taken by means of peripheral venesection using two Greiner Z-serum clot activator tubes.

Urine samples (20ml) were collected by means of a midstream clean catch urine specimen into a sterile universal container.

All samples were taken in the fasting state (> 6 hours). Patients were allowed to drink water only in this timeframe.

3.10.1 Surgery Group

Samples of blood and urine were taken on the morning of surgery, and repeat samples were taken when patients attended for routine follow-up at the surgical out patient clinic ~ 8 weeks after their procedure.

3.10.2 Biological Therapy Group

Samples of blood and urine were taken immediately prior to the first dose of biological therapy, and repeated immediately prior to their second dose 2 weeks later.

3.10.3 Treatment Naïve Group

Samples were taken immediately prior to commencing medical therapy. Patients then joined the longitudinal sampling group and had 3 monthly samples taken for the duration of 1 year.

3.10.4 Longitudinal Sampling Group

Samples of blood and urine were taken at 3 monthly intervals for the duration of 1 year.

3.10.5 Healthy Control Group

Samples were taken on the morning of elective non-gastrointestinal surgery.

3.11 Sample Preparation

Initial sample preparation and storage prior to transfer was carried out in the Department of Diabetes & Cardiovascular Science at the University of the Highlands and Islands in Inverness.

3.11.1 Blood Samples

Samples were allowed to clot at 4°C on ice for >60 mins but <120mins. Centrifugation was then carried out at 2500g for 15 minutes at 4°C. The cap of the tube was removed and, without disturbing the pellet, 0.5ml aliquots of serum were transferred, using a Pastor pipette into labelled 2ml Greiner cryovials. Care was taken to ensure that no red blood cells were carried over with the serum. The cryovials were placed in cryoboxes and stored immediately at -80°C.

3.11.2 Urine Samples

Samples were centrifuged at 5000g for 10 minutes at 4°C and then, without disturbing any precipitate, 0.5ml aliquots were transferred, using a Pastor pipette into labelled 2ml Greiner cryovials. The cryovials were placed in cryoboxes and stored immediately at -80°C.

3.12 Sample Transfer

Samples were transferred on dry ice to the Biochemistry Department of Manchester Institute of Biotechnology, where metabolomic profiling was performed on these samples under the supervision of Dr N Rattray, Dr D Trivedi and Professor R Goodacre. Here samples were analysed using both an Ultra High Performance Liquid chromatography-Fourier Transform Mass spectrometry process (UHPLC-FTMS) and a Gas chromatography-Time of Flight-Mass spectrometry process (GC-ToF-MS).

3.13 Established Methodology Utilised

In this metabolomics study established workflow protocols created by the Manchester Centre for Integrative Systems Biology, School of Chemisty, Manchester Institute of Biotechnology were used. The quality assurance protocols and sample processing steps were followed as previously defined by this expert group, thus there was no requirement for preliminary experiments to check the protocols (Dunn, Broadhurst et al. 2011).

3.14 Materials

All materials were purchased from Sigma-Aldrich (Gillingham, U.K.) unless otherwise stated. Pyridine (extra dry), hexane, methoxylamine hydrochloride, and N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) were obtained from Acros Organics (Loughborough, U.K.). The

internal standards lysine-d4, succinic acid-d4, and glycine-d5 were purchased from Sigma-Aldrich (Gillingham, U.K.). HPLC-MS grade Methanol was purchased from Fisher Scientific, UK.

3.15.1 Serum Sample Pre-treatment

Serum samples were thawed on ice (4°C) and thereafter 200µl of sample deproteinised by adding 600µl of methanol and vortex mixed for 15 secs and the centrifuged for 15 minutes at 13,500 *g*. Two aliquots of supernatant were transferred to pre-labelled eppendorf tubes - one each for GC-ToF-MS and UHPLC-FTMS analysis. Prior to drying down, all samples had 10 µL removed and pooled to provide a quality control (QC) sample whilst those only allocated for GC-ToF-MS analysis had 100µL of a 30mg mL^{-1} internal standard solution containing lysine-d_4, succinic acid-d_4, and glycine-d_5 added. Samples were then lyophilised (Centrifugal vacuum evaporator (Eppendorf 5301)) for 16hrs and sample pellets stored at -80°C until analysis.

3.15.2 Urine Sample Pre-treatment

Urine samples were thawed on ice at (4°C) and two aliquots of 100 µl were transferred to pre-labelled Eppendorf tubes – one each for GC-ToF-MS and UHPLC-FTMS analysis. From GC-ToF-MS aliquot high urea content was removed via enzymatic degradation using 150 µL urease (Urease from Canavalia ensiformis, Sigma Aldrich, U1501; 20mg mL-1, 37°C for 30mins). Subsequent addition of 400µL of methanol in both aliquots (vortexed for 15s and centrifuged at 13500 *g* for 15 minutes) resulted in protein precipitation.

100 µl supernatant was then transferred to pre-labelled Eppendorf tubes – one each for GC-ToF-MS and UHPLC-FTMS analysis. Prior to drying down, all samples had 10µL removed and pooled to provide a QC sample whilst those only allocated for GC-ToF-MS analysis had 100 µL of a 30mg mL^{-1} internal standard solution containing lysine-d_4, succinic acid-d_4, and glycine-d_5 added. Samples were then lyophilised (Centrifugal vacuum evaporator (Eppendorf 5301)) for 16hrs and sample pellets stored at -80°C until analysis.

3.16.1 Sample Preparation Prior to UHPLC-FTMS Analysis

Prior to analysis samples were reconstituted in HPLC grade water (free from organic and inorganic compounds, with no ultraviolet absorbance) (100µl per sample), vortex mixed and centrifuged for 15 minutes at 13,500 *g*. Each sample extract was transferred to a single analytical vial with 200 µL fixed insert, caped, stored in the autos ampler at 5 °C. Samples were analysed within 48h of reconstitution in positive ionisation mode. The samples were analysed over four separate analytical blocks each completely randomised.

3.16.2 UHPLC-FTMS Conditioning, Calibration and Tuning

All samples were analysed on the Accela UHPLC system (Thermo-Fisher Ltd. Hemel Hempsted, U.K.) coupled to an electrospray LTQ-Orbitrap XL hybrid mass spectrometry system (ThermoFisher,

Bremen, Germany). UHPLC columns were conditioned with solvent B (HPLC-MS grade methanol and 0.1% formic acid) at 50°C, gradually increasing the flow rate to 400 µL.min^{-1} over 30 mins. Thereafter a 50:50 solution solvent A (HPLC water and 0.1% formic acid) and solvent B was used for 3 hours in order to condition column. The column was maintained at 95:5 Water: MeOH composition until analysis begun.

Prior to sample analysis, the LTQ-Orbitrap MS was tuned to optimise detection of ions in the *m/z* 50-1000 range and calibrated according to the manufacturers predefined methods using ultra mark and caffeine mixture. Data were acquired in the Orbitrap mass analyser operating at a mass resolution of 30,000 (FWHM defined at *m/z* 400) and a scan speed of 0.4 s. Prior to and in-between each analytical block the ESI ion tube and spray deflector were cleaned using 8:2 HPLC grade methanol: water acidified with 1 % formic acid and ultra-sonication for 15 minutes.

3.16.3 UHPLC-FTMS Parameters

Analysis was carried out on an Accela UHPLC auto sampler system using a Hypersil Gold C_{18} reverse phase column (L = 100mm, D = 2.1 mm, particle size 1.9 µm) coupled to an electrospray LTQ-Orbitrap XL hybrid mass spectrometry system (ThermoFisher, Bremen, Germany). Excalibur and TunePlus software were used for instrument operation and tuning and calibration was carried out as per the manufactures instruction. 10 µL of each sample was injected on to the column and a methanol/water solvent gradient below was used for metabolite separation over the column.

Table 3.5: UHPLC-FTMS Solvent Gradient for Reverse Phase Analysis

Time (min)	Flow Rate (µLmin^{-1})	Mobile Phase A (H_2O%)	Mobile Phase A (MeOH%)
Initial	400	100	0
5	400	100	0
15	400	0	100
25	400	0	100
30	400	100	0

3.16.4 Orbitrap Parameters

- 1 micro scan per 400ms, 100-1000 *m/z* range
- ESI ion source transfer tube set at 275°C
- Tube lens voltage = 100V (-90V in negative mode)
- Capillary V = - 30V (-28V in negative mode)
- Sheath Gas = 40, Aux Gas =5, Sweep Gas = 1
- Resolution = 30000 in centroid

3.16.5 Processing of Raw UHPLC-FTMS Profiles and Analyte Identification

The UHPLC-FTMS raw data profiles were converted into a NetCDF format within the Xcalibur software's file conversion programme. Peak deconvolution was performed using XCMS software (http://masspec.scripps.edu/xcms/xcms.php) with in-house script. The XCMS deconvolution produced an MS Excel based XY matrix of mass spectral features (with related accurate *m/z* and retention time variable pairs) *x* sample, with peak area input where the mass spectral feature was detected in each sample.

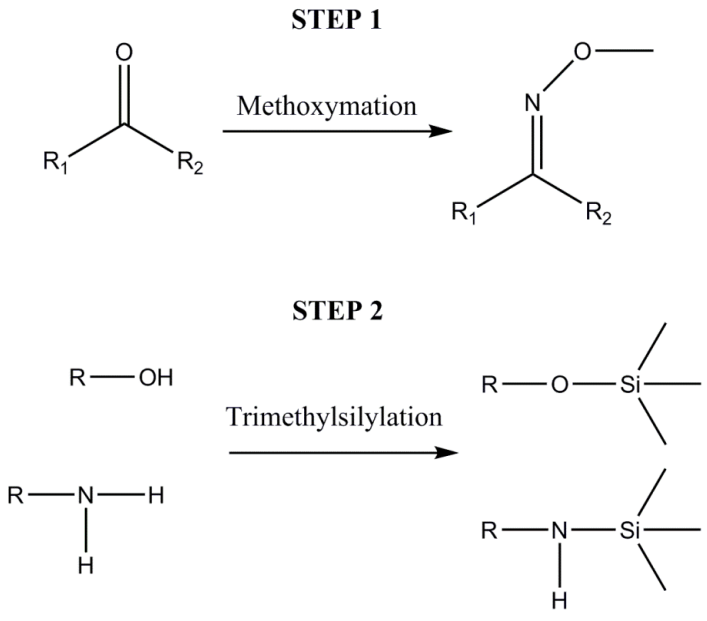
Peaks that failed quality control (greater than 20% RSD (relative standard deviation) within analytical QCs) were removed and not analysed further. The putative identification of features detected on the UHPLC-FTMS platform was performed by accurate mass match (*m/z* tolerance of 0.01) with Human Metabolome Database (www.hmdb.ca)

3.17.1 Sample Preparation Prior to GC-ToF-MS Analysis

Prior to analysis via GC-ToF-MS, a two-stage chemical derivatisation process was carried out to impart volatility to non-volatile metabolites. Methods are briefly summarised as follows:

The process of derivatisation modifies metabolites not volatile enough to be analysed within a GC-ToF-MS system with methoxy and trimethylsilyl groups (TMS). These groups help analytes to remain in the gas phase and enable separation on a GC column. The methoxy group is used to protect carbonyl moieties, such as ketones, and to reduce sugars in order to open cyclic ring structures. The relative chemical inertness and lack of presence within nature allows the TMS grouping to act as an efficient GC derivatisation agent within the two step process:

Figure 3.2: Reaction Scheme Representing the Two Chemical Transformations of GC Derivatisation



The first reaction, oxymation, was performed by heating previously prepared sample pellets in the presence of 50 µL of O-methoxyamine (20mg/mL in pyridine) for 40 mins at 65°C. Then the second stage trimethylsilyation was performed where samples were reacted with 50 µL of MSTFA (N-acetyl-N-(trimethylsilyl)-trifluoroacetamide) for 40mins at 65°C. Derivatised samples then had 20 µL of a retention index mixture added (30mg/mL dodecane, pentadecane, nonadecane, docosane at 30mg/mL dissolved in hexane and diluted 1:10 in pyridine), were centrifuged for 15 minutes at 13500 *g* with the resultant supernatant added to appropriately labelled GC-MS amber vial with fitted 200 µL insert. To minimise degradation of sample at room temperature while sitting in the auto sampler, each sample was analysed within a 48h window.

For each analytical block, initially 10 injections of pooled QC sample were performed for column conditioning, after which 5 injections of experimental samples were made, followed by a QC injection. This was repeated until all samples within the block were analysed. Finally three QC injections were made at the end of the block run.

3.17.2 GC-TOF-MS Analysis

All samples were analysed on an Agilent 6890 GC (Agilent Technologies, Stockport, UK) coupled to a LECO Pegasus III electron impact mass spectrometer (LECO Corp., St. Joseph, MO) with appropriate instrument pre-operation checks.

Initially 10 QC samples were run before any sample injection to condition the column. Samples were injected through injector into inlet 280°C helium carrier flow at a rate of 1ml min^{-1}. A DB5-MS column (Supelco, Gillingham, UK, 30m x 0.25mm x 0.25µm film thickness) was used with the transfer line held at 230°C. Samples were analysed within a mass range of 30-600 Da at a detector voltage of 1550 V. Temperature programme was as follows: 4 minute hold at 70 °C, then 20 °C/min increase to reach 300°C, followed by a 4 min hold. Experimental samples were interspersed with regular pooled QC samples to assess drift.

3.17.3 Processing of Raw GC-TOF-MS Profiles and Metabolite Identification

The raw data profiles were converted into a NetCDF format within LECO ChromaTof v2.32. Peak deconvolution was performed using XCMS software (http://masspec.scripps.edu/xcms/xcms.php) with in-house script. The XCMS deconvolution produced an MS Excel based XY matrix of mass spectral features (with related accurate m/z and retention time variable pairs) x sample, with peak area input where the mass spectral feature was detected in each sample. Peaks that failed quality control (greater than 30% RSD within analytical QCs) were removed and not analysed further. Putative identification was carried out by matching spectra and retention times with in-house library spectra with help of retention indices as well as National Institute of Standards and Technology (NIST) libraries.

3.18 Data Pre-processing

Deconvolved data was normalised by total ion count followed by log transformation (glog) and auto scaling (mean-centered and divided by the standard deviation of each variable). For various research questions of interest, the data was re-organised using one-way analysis of variance (ANOVA), and then Mann-Whitney U test as well as Wilcoxon rank sum test were performed. Metabolites with p-values < 0.05 were considered significant. These p-values were then corrected using Bonferroni correction (q value). The resultant variables identified with p-value and q-value < 0.05 were considered to be truly significant without any bias.

When considering large data sets with multiple testing, such as the longitudinal sampling group, Bonferroni correction can be too conservative, reducing the number of false positives, but also the number of true discoveries, and therefore p values were corrected using an optimised False Discovery Rate (FDR) approach. This approach is designed to control the expected proportion of rejected null hypotheses that were incorrect rejections, and was used in correlation analysis where fewer features are significantly correlated. When considering comparisons between two conditions where hundreds of metabolites are significant, Bonferroni tends to be a stricter criteria leading to fewer significant features that stand the correction procedure. Throughout the results it is stated when Bonferroni or FDR corrections are utilised.

PCA correlation analysis was performed with diseases scores and patient metabolite profiles. Classification of samples into groups was tested using PLS-DA analysis validated by bootstrapping.

Metabolite lists thus obtained, were then assigned biologically relevant IDs, and box-plots were created to visualise the difference.

When utilising paired samples, differences in the variables deemed significant were visualised by calculating the numeric difference between process peak areas and plotting bar charts.

3.19 Power Calculation

This is the first study in the UK of its kind in view of the logitundinal sampling taking place. No power calculation was required as part of this novel project, as this is a hypothesis generating study and thus no sample size can be reliably determined. It has been suggested that 20-30 participants is appropriate to control for variance between samples in a feasibility or pilot study (Lancaster, Dodd et al. 2004). Johnston et al (Johnston 2014) sampled 30 CD patients, 30 UC patients and 30 healthy controls in their study. These numbers allowed the generation of metabolomic data that was able to differentiate between the groups studied. Based on these observations and the numbers of patients thought to be available for recruitment we designed the study.

3.20 Declaration of Assigned Work

Due to the specialised nature of metabolomic profiling, assistance was required for data analysis of samples utilising both the UHPLC-FTMS and GC-ToF-MS platforms. Bioinformatic analysis of raw data to identifiy differentiation of metabolomic profiles between the study groups, as well as the identification of significant differentiating metabolites was carried out by the team at MIB.

The study design, patient recruitment, data collection, sample collection and sample preparation were performed by me. I carried out the work on interpretation and relevance of identified metabolites, and their clinical importance to disease processes.

4

Results

4.1 Recruitment Data

In total, 84 patients with IBD were recruited over an 18 month period; 41 with UC, 43 with CD, and 62 HC. All were from Raigmore Hospital, Inverness. All of the IBD patients were recruited by me. The HC were recruited by me and Natasha Ross, Clinical Research Fellow.

Sample sets of blood and urine were taken as previously discussed. During the study it was not possible to take blood from 1 IBD patient, and 2 IBD patients and 2 healthy controls failed to pass urine during the required timeframe.

4.2 Participant Demographics

A total of 255 samples sets were taken from IBD patients by me. Of the IBD patients analysed, 164 sample sets were from females, and 91 sample sets from males. The median age at the time of sampling was 44 years (range 19 – 77, IQR 33 - 58). The median age in the UC group was 46 years (IQR 33 – 58), and the median age was 42 years (IQR 34 – 53.8) in the Crohn's Disease group.

All of the IBD participants were of Caucasian origin, however, of these there was one Australasian participant who moved to the UK in 2010, and one Southern European participant who had lived in Scotland for >10 years.

The IBD participants had a mean BMI of 26.17 (range 15.45 – 38.67), with a median of 25.2 (IQR 23.2 – 28.9). UC participants had a median BMI of 25.6 (IQR 24.4 – 28.4) and CD participants a median BMI of 24.3 (IQR 22.0 – 30.2).

17% of IBD sample sets were taken from current smokers, 41% from ex-smokers, and 42% from those who have never smoked. In UC, 11% of samples were taken from current smokers compared to 23% of CD samples ($p<0.05$). In UC, 48% of samples were from ex-smokers and 41% from non-smokers. In CD, 33% of samples were from ex-smokers, and 43% from non-smokers.

92% of samples were taken from those eating a normal diet, 4% of samples were taken from vegetarians, 2% of samples were taken from participants who do not eat red meat, 1% from participants on a low residue diet, 1 sample set from a gluten free patient and 1 sample set from a participant receiving total parental nutrition.

During the study three patients died, one in the surgical group, and one in the biological and longitudinal groups. One other patient, from the surgical group, withdrew from the study following a diagnosis of cholangiocarcinoma, and died during the study period. One patient withdrew from the longitudinal group, one was not contactable following the second set of longitudinal samples, and one was withdrawn when she became pregnant.

A total of 62 sample sets were taken from healthy controls. Of the HCs 48 sample sets were from males and 14 from females. The median age at the time of sampling was 53 years (range 28 – 83 years). The mean BMI was 27.3 (range 18.5 – 43.6), median BMI 26.3 (IQR 24.1 – 29.9). All of the HCs were British Caucasian apart from one Eastern European Caucasian. 72% were non-smokers, 15% ex-smokers and 13% current smokers. There was one vegetarian HC and one HC with alcohol excess.

4.2.1 Surgery Group

A total of 30 participants undergoing surgical procedures for the management of IBD were recruited by me. Both elective and emergency patients were included. Of the 30 patients recruited, 15 had UC and 15 had CD. 10 patients underwent a colectomy, 6 small bowel resections, 4 completion proctectomy, 3 perianal procedures, 3 ileocaecal resections +/- stoma formation, 2 panproctocolectomy and 2 right hemicolectomy. 28 complete serum samples sets and 27 complete urine sample sets were available for paired sample analysis.

4.2.2 Biological Group

A total of 24 patients receiving biological therapy were recruited to the study. 14 patients had Crohn's disease and 10 patients had UC. 21 complete samples sets were available for paired sample analysis.

4.2.3 Treatment Naïve Group

Six treatment naïve patients were recruited. One was excluded from the study when he was found to have he was identified as having a diverticular mass rather than a new diagnosis of Crohn's disease.
Of the five remaining patients 3 had CD and 2 had UC.

4.2.4 Longitudinal Group

Forty patients were recruited. 22 patients had UC and 18 had CD.

4.2.5 Healthy Controls

In total, 63 patients were recruited and 62 patient samples were analysed. One patient was excluded as they were found to have a daughter with IBD, which was only established after sampling.
Of note, the healthy control group was older and there were more females than in the IBD groups.

Table 4.1: Participant Demographics

	UC (n=41)	CD (n=43)	HC (n=62)	p value
Sample sets	127	129	62	
Median Age in years (range)	46 (19 – 77)	43 (20 – 73)	53 (40 – 62)	0.001***
Sex M:F ratio	2.26:1	1.43:1	3.42:1	<0.0001**
Ethnicity	39 British Caucasian 1 Southern European Caucasian 1 Australasian Caucasian	43 British Caucasian	61 British Caucasian 1 Eastern European Caucasian	1**
BMI (median)	25.6 (IQR 24.4 – 28.4)	24.3 (IQR 22.0 – 30.2)	26.3 (IQR 24.1 – 29.9)	0.18***
Current Smoker **Ex Smoker** **Non Smoker**	11% 48% 41%	23% 33% 43%	13% 15% 72%	0.05**
Diet	1 no red meat 3 vegetarian	2 low residue 1 TPN 1 gluten free 1 no red meat 1 vegetarian	1 vegetarian	0.64**
Alcohol excess*	1	2	1	0.64**

*Alcohol excess defined as > 14 units for a female or > 21 units for a male per week.

** Fisher's exact test

*** Independent t-test

The complete table of metadata from the study participants is presented in appendix 8.2.

The sample collection details for the IBD patients are presented in appendix 8.3.

4.3 Experiment 1: Differentiation HC v UC v CD

4.3.1 Experiment 1 Aims

To determine whether it is possible to differentiate between the metabolomic profiles of healthy controls, ulcerative colitis patients and Crohn's Disease patients.

Analyses are carried out on serum and urine samples, and using GC-ToF-MS, and UHPLC-FTMS platforms.

Table 4.2 Experiment 1 number of samples analysed

	Heathly Controls	**UC**	**CD**
Serum samples	62	127	128
Urine samples	60	125	127

Confusion matrices and bar charts are used to visualise the results. Identification of metabolites and their biological relevance is considered in future experiements.

A confusion matrix is a table used to describe the performance of a classification model on a set of test data.

Table 4.3: Example of a confusion matrix

		Predicted	
		No	Yes
Actual	No	60	40
	Yes	25	75

There are two possible predicted classes in the table: "yes" and "no". A total of 200 predictions are made in this example (e.g. 200 patients being tested for a disease), and of these the classifier predicted "yes" 115 times, and "no" 85 times. The actual number of "yes" is 100, and "no" is 100, therefore in reality 100 patients have the disease and 100 do not. The true positives are the cases predicted "yes" that have the disease. The true negatives and those predicted "no" that do not have the disease. False positives are those predicted "yes" that do not have the disease (type I error), and false negatives are those predicted "no" who do have the disease (type II error). The percentage of cases correctly classified, the correct classification rate (CCR) is recorded. The confusion matrix is visualised using a colour map with the rows representing the x-axis representing the predicted label and the y-axis representing the actual label.

Bar charts are used to compare the null hypothesis, that there is no discerable difference between HCs, CD and UC, and the observed profile.

4.3.2 Experiment 1.1 Results

Figure 4.1: Visualisation of Confusion Matrix of GS-ToF-MS Serum Analysis HC v UC v CD

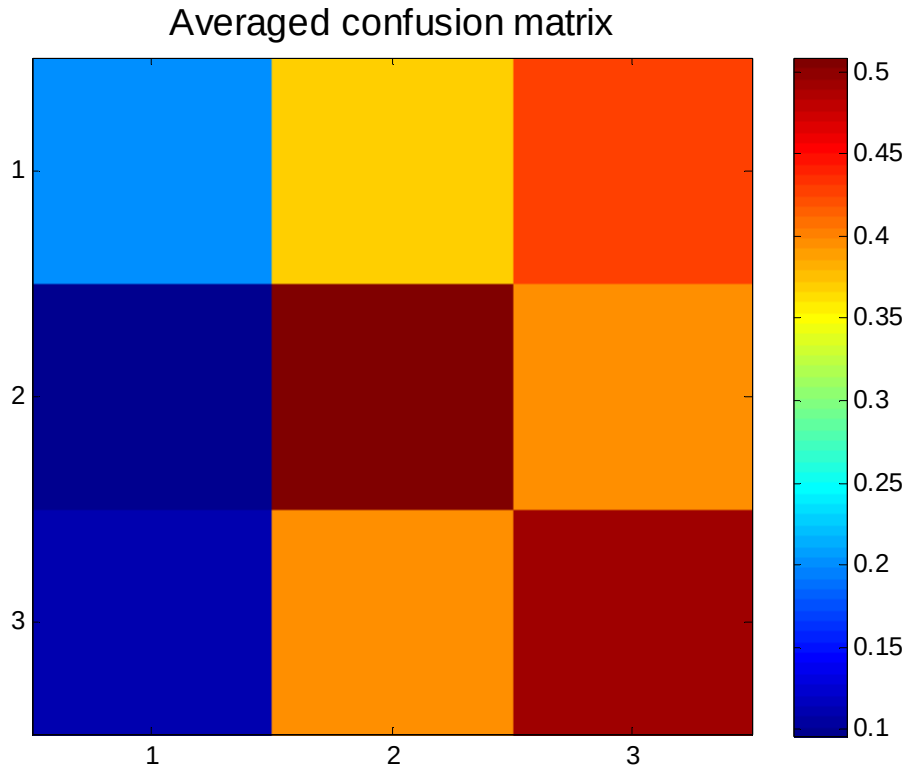


Table 4.4: Confusion Matrix of GS-ToF-MS Serum Analysis HC v UC v CD

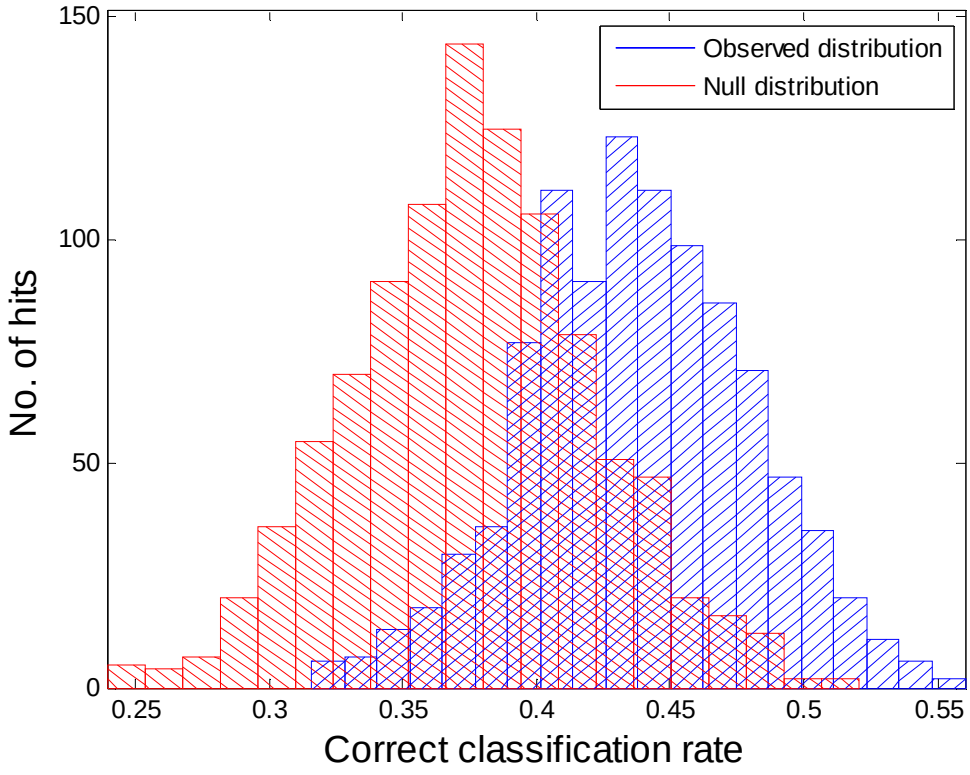
		Predicted		
		1 (HC)	2 (UC)	3 (CD)
Actual	1 (HC)	20.0%	37.0%	43.0%
	2 (UC)	9.6%	50.9%	39.5%
	3 (CD)	11.4%	39.5%	49.1%

Averaged CCR% = 43.7%

The averaged correct classification rate is 43.7%, with UC being the correctly identified as UC most frequently: in 50.9% of cases. CD is correctly identified as CD in 49.1% of cases. It appears difficult to differentiate HCs from IBD as in 37% of actual HC cases UC is predicted, and in 43% of cases CD is predicted. However, it is less common for actual cases of UC or CD to be predicted as a healthy control (9.6% and 11.4% of cases respectively). Differentiating between UC and CD is challenging in this experiment with 39.5% of actual UC cases being predicted as CD, and 39.1% of actual CD cases being predicted as UC.

The bar chart shows that the observed distribution of the profile is not significantly different to the null distribution ($p>0.1$).

Figure 4.2: Bar Chart of GS-ToF-MS Serum Analysis HC v UC v CD Observed and Null Distributions



p-value > 0.1

In this dataset, using serum samples analysed by GS-ToF-MS, it is not possible to differentiate between HCs, CD and UC.

4.3.3 Experiment 1.2: Results

Figure 4.3: Visualisation of Confusion Matrix of UHPLC-FTMS Serum Analysis HC v UC v CD

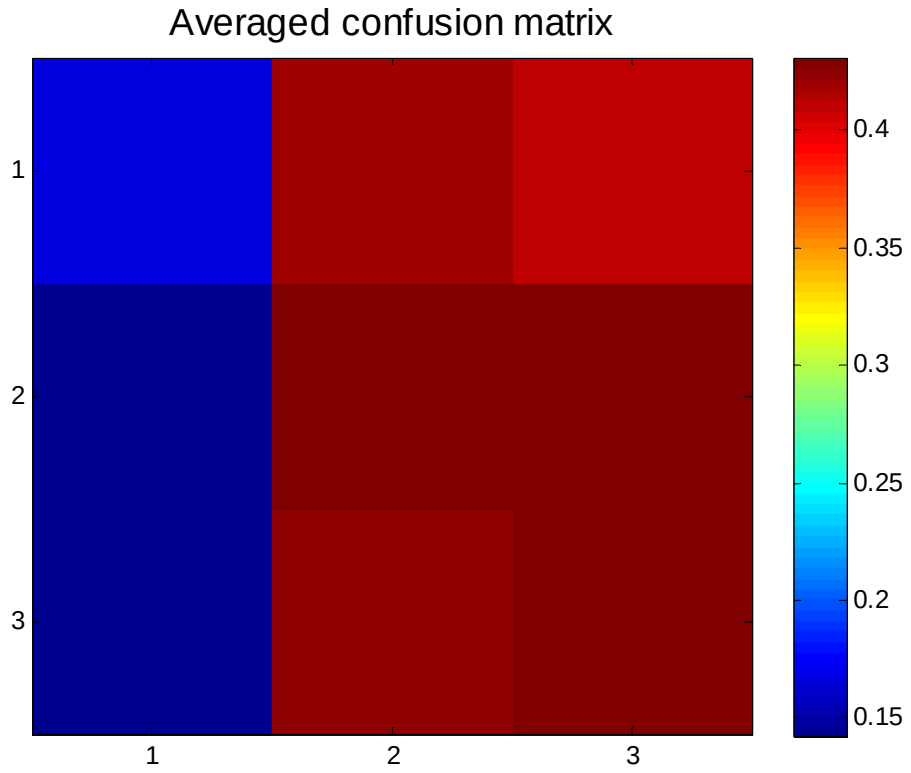


Table 4.5: Confusion Matrix of UHPLC-FTMS Serum Analysis HC v UC v CD

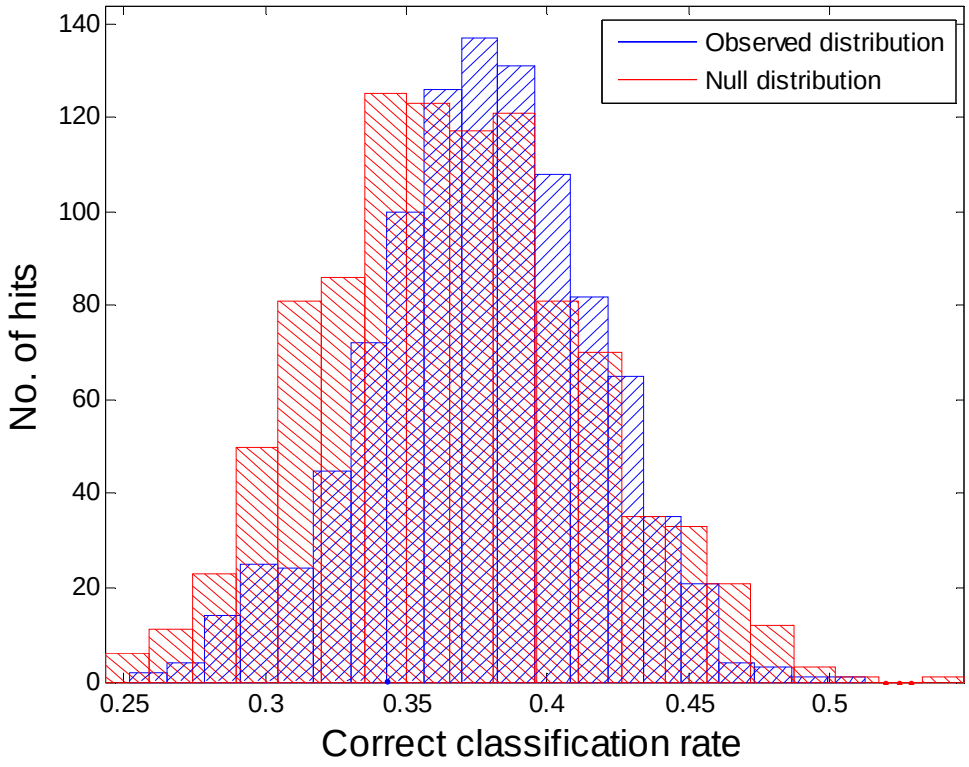
		Predicted		
		1 (HC)	2 (UC)	3 (CD)
Actual	1 (HC)	16.9%	42.0%	41.0%
	2 (UC)	14.2%	43.1%	42.7%
	3 (CD)	14.6%	42.4%	43.0%

Averaged CCR% = 38.6%

The averaged correct classification rate is 38.6%. Actual cases of HCs are only correctly classified in 16.9% of cases in this analysis. Whilst actual cases of UC and CD are only predicted as HCs in 14.2% and 14.6% of cases respectively, it is not possible to correctly differentiate between UC and CD, with true positives in UC in only 43.1% of cases, and in CD in 43% of cases.

The bar chart shows that the observed distribution of the profile is not significantly different to the null distribution ($p>0.1$).

Figure 4.4: Bar Chart of UHPLC-FTMS Serum Analysis HC v UC v CD Observed and Null Distributions



p-value > 0.1

In this dataset, using serum samples analysed by UHPLC-FTMS, it is not possible to differentiate between HCs, CD and UC.

4.3.4 Experiment 1.3: Results

Figure 4.5: Visualisation of Confusion Matrix of GC-ToF-MS Urine Analysis HC v UC v CD

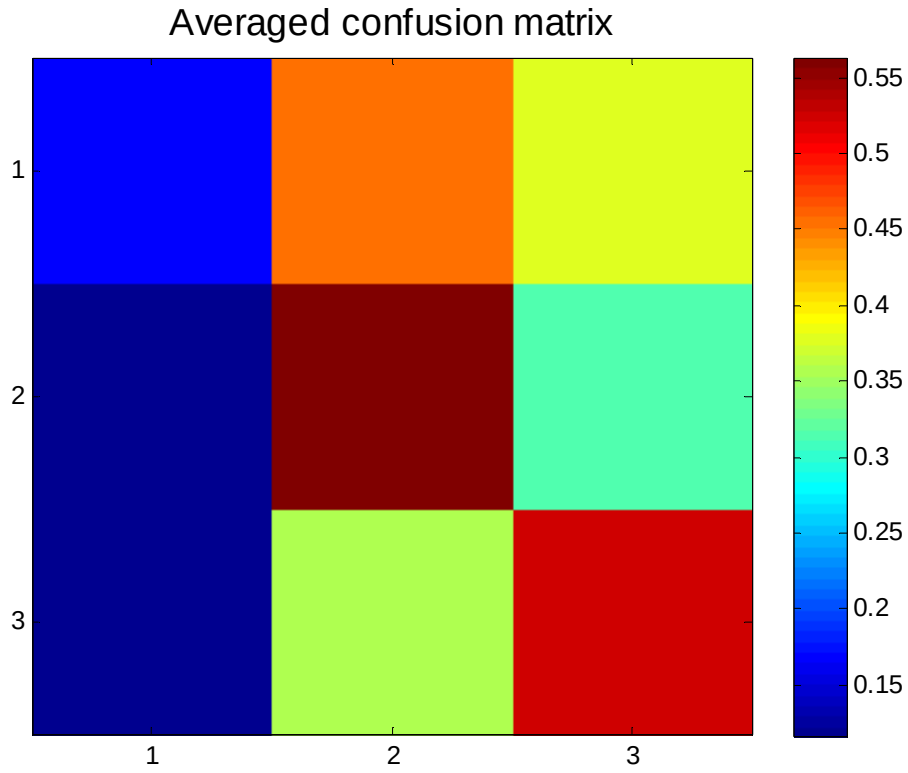


Table 4.6: Confusion Matrix of GC-ToF-MS Urine Analysis HC v UC v CD

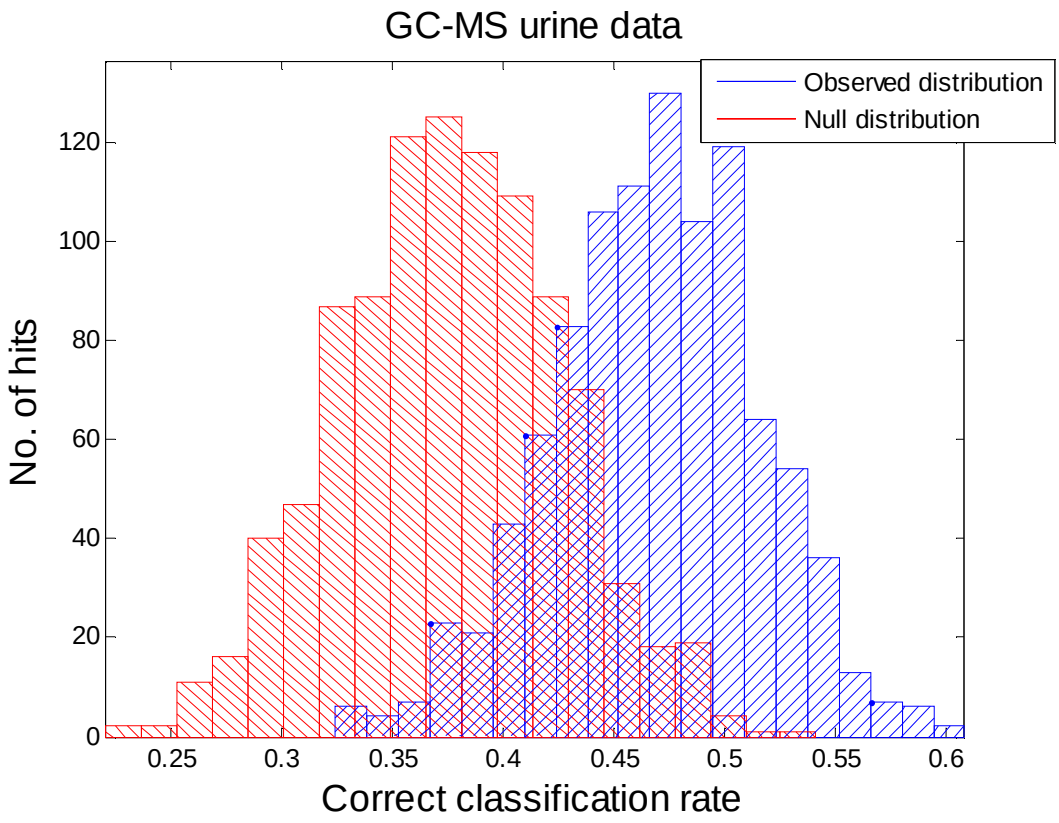
		Predicted		
		1 (HC)	2 (UC)	3 (CD)
Actual	1 (HC)	16.8%	45.3%	37.9%
	2 (UC)	12.3%	56.3%	31.4%
	3 (CD)	11.6%	35.7%	52.6%

Averaged CCR = 46.9%

The averaged correct classification rate is 46.9%. In this dataset it is possible to correctly predict UC in 56.3% of cases and CD in 52.6% of cases. Actual cases of UC and CD are only predicted as HC in 12.3% and 11.6% of cases respectively, however, actual HCs are predicted to be UC in 45.3% of cases, and CD in 37.9% of cases.

The bar chart shows that the observed distribution of the profile is not significantly different to the null distribution; however, it shows more differention from the null distribution than in Experiment 1.1 and 1.2 ($p<0.1$).

Figure 4.6: Bar Chart of GC-ToF-MS Urine Analysis HC v UC v CD Observed and Null Distributions



p-value < 0.1

In this dataset, using urine samples analysed by GC-ToF-MS, it is not possible to differentiate between HCs, CD and UC. However, this dataset on this platform shows more differentiation than seen in the serum analyses.

4.3.5 Experiment 1.4: Results

Figure 4.7: Visualisation of Confusion Matrix of UHPLC-FTMS Urine Analysis HC v UC v CD

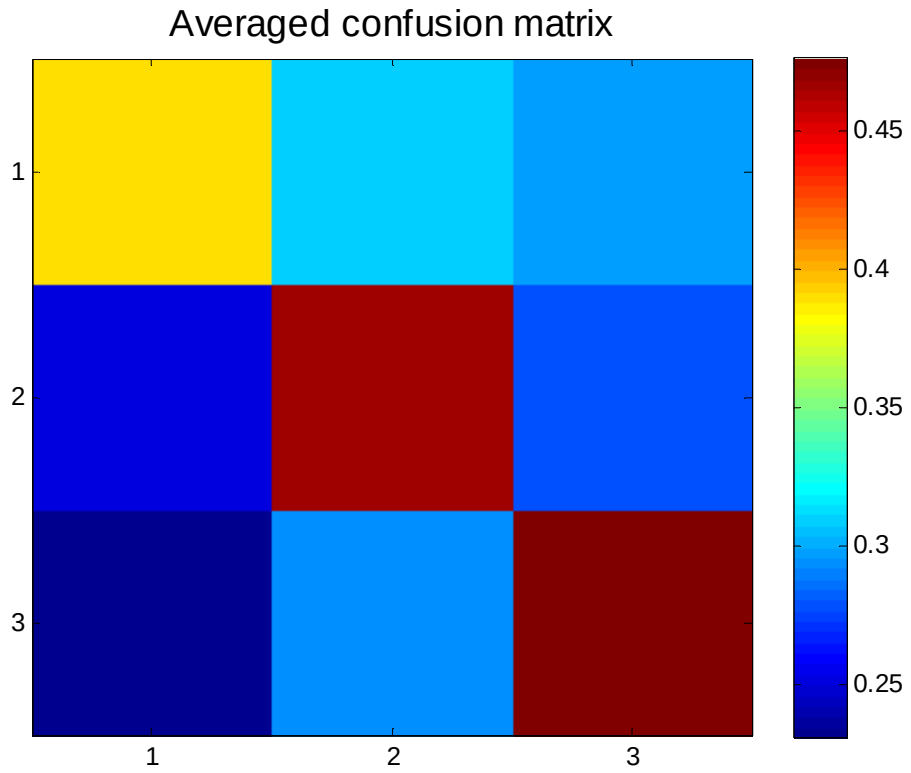


Table 4.7: Confusion Matrix of UHPLC-FTMS Urine Analysis HC v UC v CD

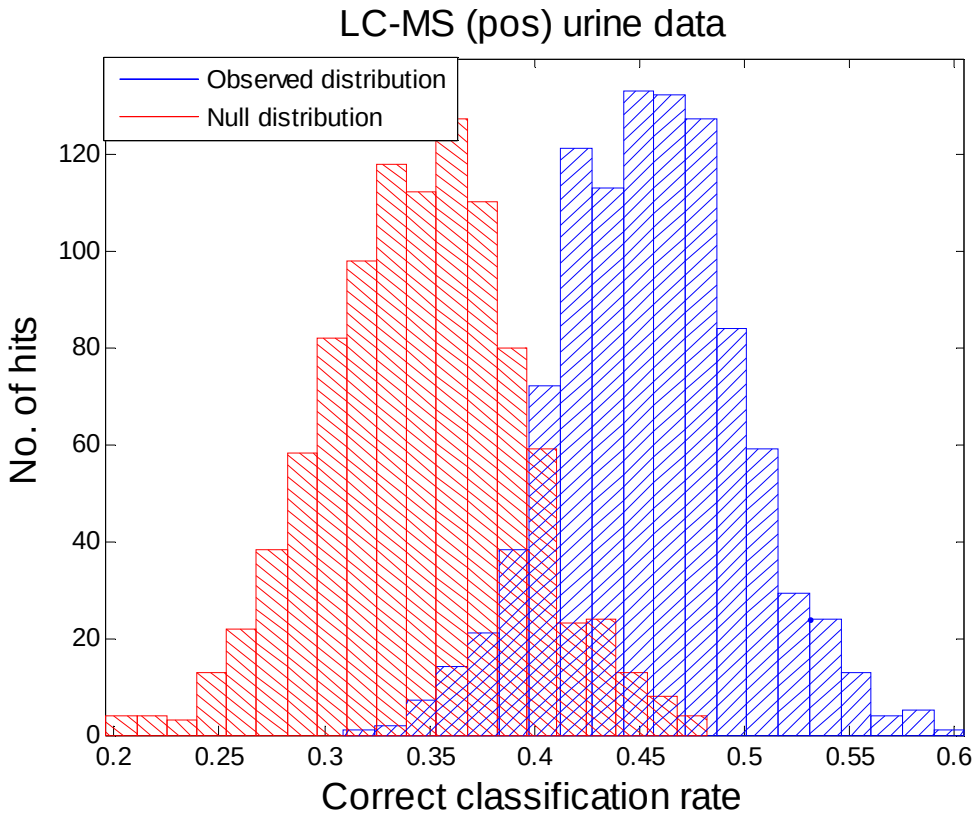
		Predicted		
		1 (HC)	2 (UC)	3 (CD)
Actual	1 (HC)	39.2%	31.1%	29.7%
	2 (UC)	25.4%	47.0%	27.6%
	3 (CD)	23.0%	29.1%	47.9%

Averaged CCR = 45.7%

The averaged correct classification rate is 45.7%. It is possible to correctly predict HCs in 39.2% of cases, UC in 47% of cases and CD in 47.9% of cases. The differentiation in this dataset appears greater between UC and CD, with only 27.6% of UC cases being predicted as CD, and only 29.1% of CD cases being predicted as UC.

The bar chart shows that the observed distribution of the profile is not significantly different to the null distribution; however, it shows more differention from the null distribution than in Experiment 1.1 and 1.2 ($p<0.1$), similar to Experiment 1.3.

Figure 4.8: Bar Chart of UHPLC-FTMS Urine Analysis HC v UC v CD Observed and Null Distributions



p-value < 0.1

In this dataset, using urine samples analysed by UHPLC-FTMS, it is not possible to differentiate between HCs, CD and UC. However, this dataset on this platform shows more differentiation than seen in the serum analyses.

4.3.6 Experiment 1 Summary

- No significant differentiation was seen between healthy controls, ulcerative colitis patients and Crohn's Disease patients when comparing either serum or urine samples on either UHPLC-FTMS (pos) or GC-ToF-MS platforms
- Urine samples appear to give greater differentiation between the groups than serum samples on both UHPLC-FTMS (pos) and GC-ToF-MS platforms
- Samples of urine analysed by GC-ToF-MS have the greatest correct classification rate (46.9%)
- Overall ulcerative colitis has the highest true positve predictive rate

4.4 Experiment 2: Pre- and Post-Surgery

4.4.1 Experiment 2 Aims

To determine whether it is possible to differentiate between the metabolomic profiles of IBD patients immediately prior to surgery and 8 weeks after surgery.

Analyses are carried out on serum and urine samples, and using GC-ToF-MS, and UHPLC-FTMS platforms.

Table 4.8 Experiment 2 number of samples analysed

	Pre-surgery	**Post-surgery**
Serum samples	30	28
Urine samples	30	28

Confusion matrices and bar charts are used to visualise the results.

Bar charts are used to compare the null hypothesis, that there is no discerable difference between the profile of pre and post surgical patients.

4.4.2 Experiment 2.1: Results

Figure 4.9: Visualisation of Confusion Matrix of GC-ToF-MS Serum Analysis Pre- and Post-Surgery

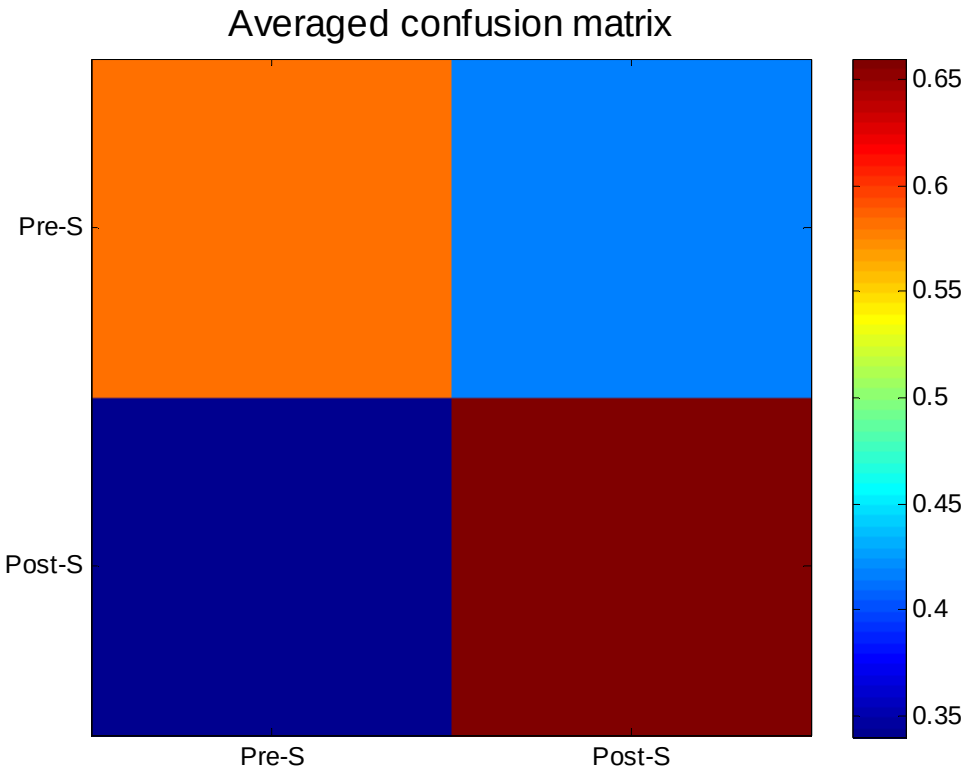


Table 4.9: Confusion Matrix of GC-ToF-MS Serum Analysis Pre- and Post-Surgery

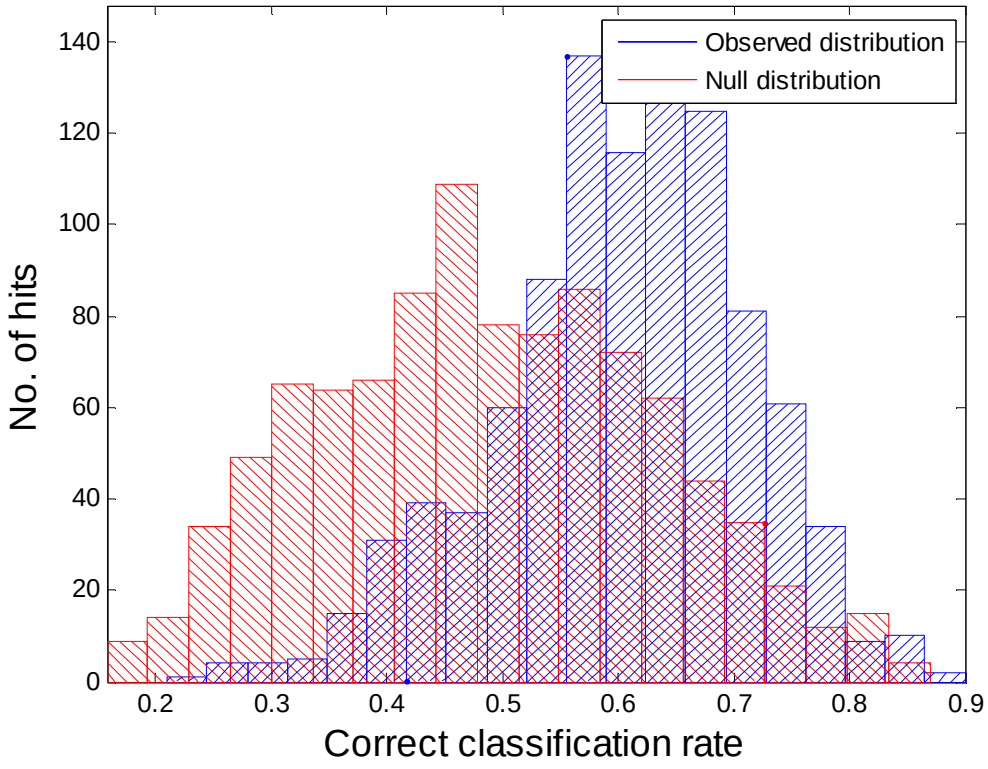
		Predicted	
		Pre Surgery	Post Surgery
Actual	Pre Surgery	58.1%	41.9%
	Post Surgery	34.0%	66.0%

Averaged CCR% = 60.3%

The averaged correct classification rate is 60.3%. In pre-surgery cases the true positive rate is 58.1% and in post-surgery cases it is 66.0%.

The bar chart shows that the observed distribution, of pre-surgery versus post-surgery, is not significantly different to the null distribution ($p>0.1$).

Figure 4.10: Bar Chart of GC-ToF-MS Serum Analysis Pre- and Post-Surgery Observed and Null Distributions



p-value > 0.1

In this dataset, using serum samples from pre- and post-surgery IBD patients, analysed by GC-ToF-MS, it is not possible to differentiate between pre-and post-surgery profiles.

4.4.3 Experiment 2.2: Results

Figure 4.11: Visualisation of Confusion Matrix of UHPLC-FTMS Serum Analysis Pre- and Post-Surgery

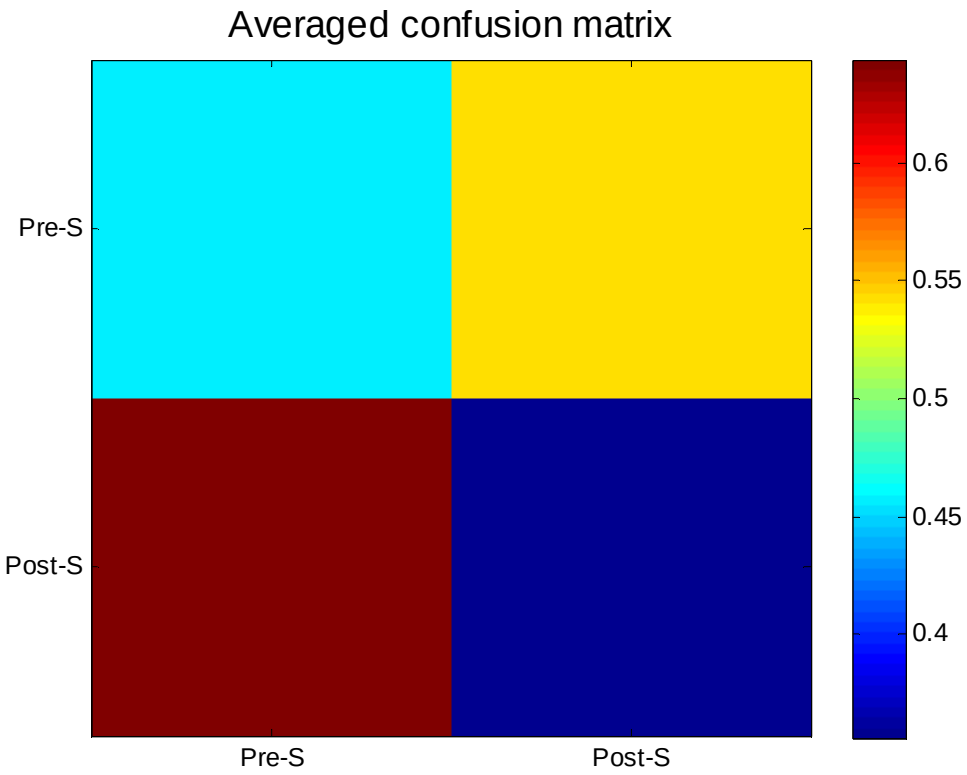


Table 4.10: Confusion Matrix of UHPLC-FTMS Serum Analysis Pre- and Post-Surgery

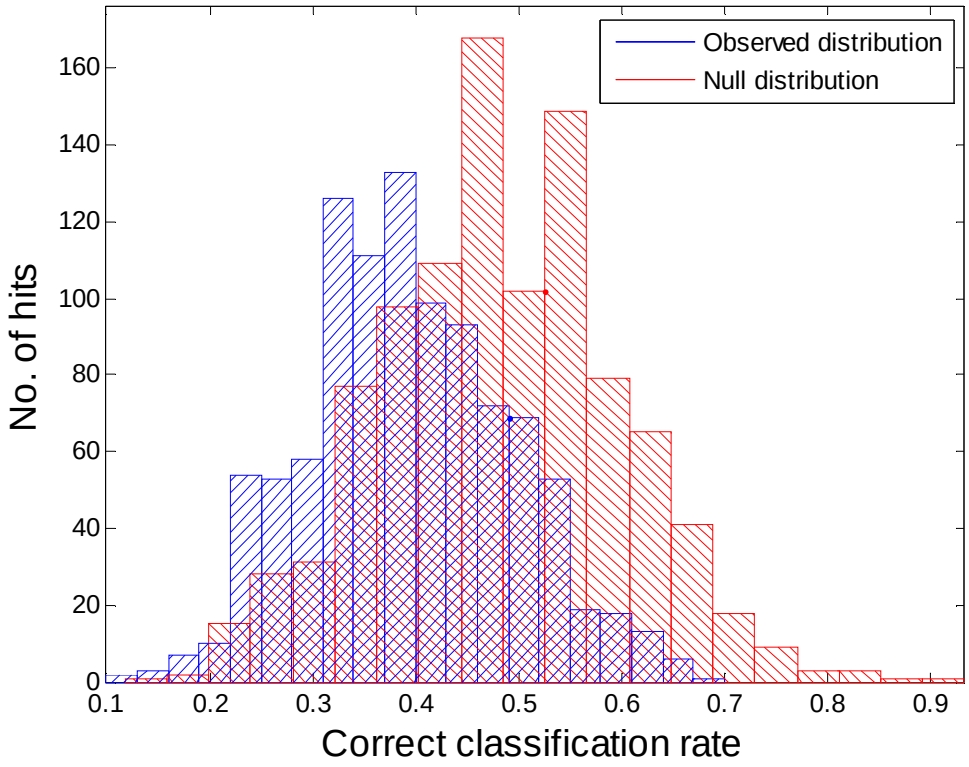
		Predicted	
		Pre Surgery	Post Surgery
Actual	Pre Surgery	45.9%	54.1%
	Post Surgery	64.4%	35.6%

Averaged CCR% = 39.7%

The averaged correct classification rate is 39.7%. It is only possible to correctly predict the pre- and post-surgery groups in 45.9% and 35.6% of cases respectively.

The bar chart shows that the observed distribution, of pre-surgery versus post-surgery, is not significantly different to the null distribution ($p>0.1$).

Figure 4.12: Bar Chart of UHPLC-FTMS Serum Analysis Pre- and Post-Surgery Observed and Null Distributions



p-value > 0.1

In this dataset, using serum samples from pre- and post-surgery IBD patients, analysed by UHPLC-FTMS, it is not possible to differentiate between pre-and post-surgery profiles.

4.4.4 Experiment 2.3: Results

Figure 4.13: Visualisation of Confusion Matrix of GC-ToF-MS Urine Analysis Pre- and Post-Surgery

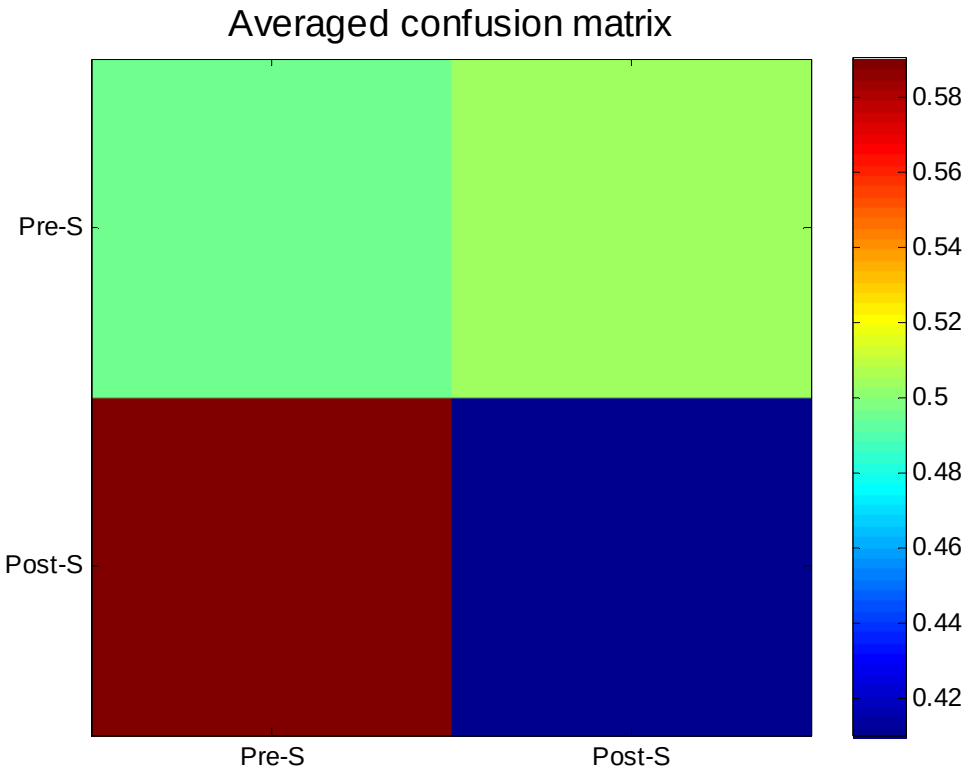


Table 4.11: Confusion Matrix of GC-ToF-MS Urine Analysis Pre- and Post-Surgery

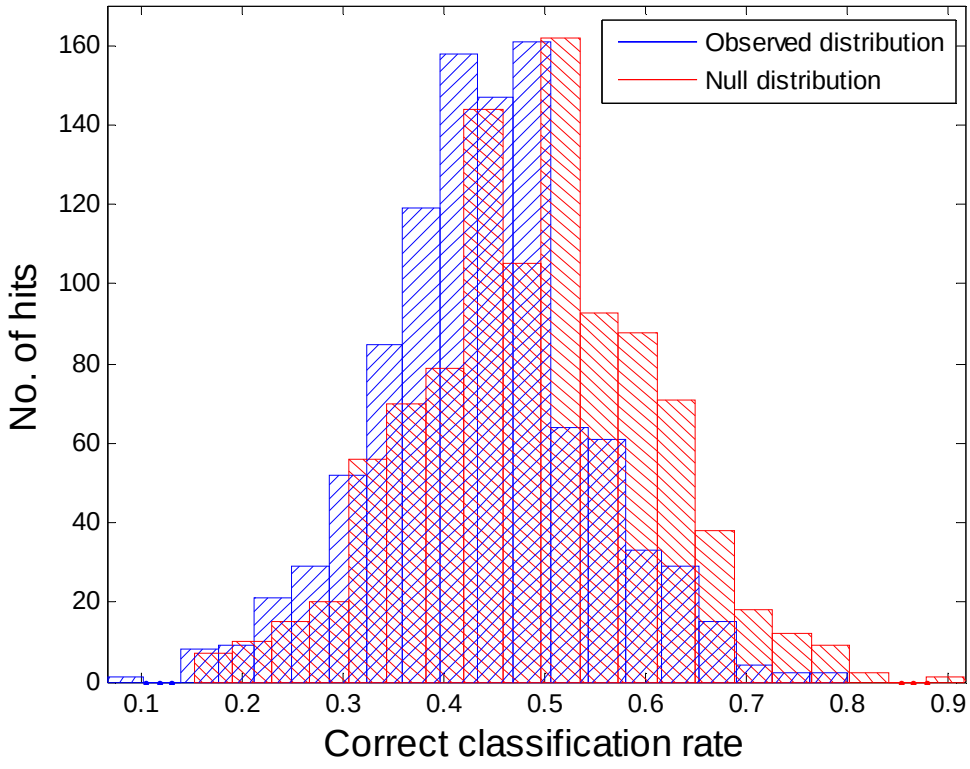
		Predicted	
		Pre Surgery	Post Surgery
Actual	Pre Surgery	49.6%	50.4%
	Post Surgery	59.0%	41.0%

Averaged CCR% = 43.8%

The averaged correct classification rate is 43.8%. It is only possible to correctly predict the pre- and post-surgery groups in 49.5% and 41.0% of cases respectively.

The bar chart shows that the observed distribution, of pre-surgery versus post-surgery, is not significantly different to the null distribution ($p>0.1$).

Figure 4.14: Bar Chart of GC-ToF-MS Urine Analysis Pre- and Post-Surgery Observed and Null Distributions



p-value > 0.1

In this dataset, using urine samples from pre- and post-surgery IBD patients, analysed by GC-ToF-MS, it is not possible to differentiate between pre-and post-surgery profiles.

4.4.5 Experiment 2.4: Results

Figure 4.15: Visualisation of Confusion Matrix of UHPLC-FTMS Urine Analysis Pre- and Post-Surgery

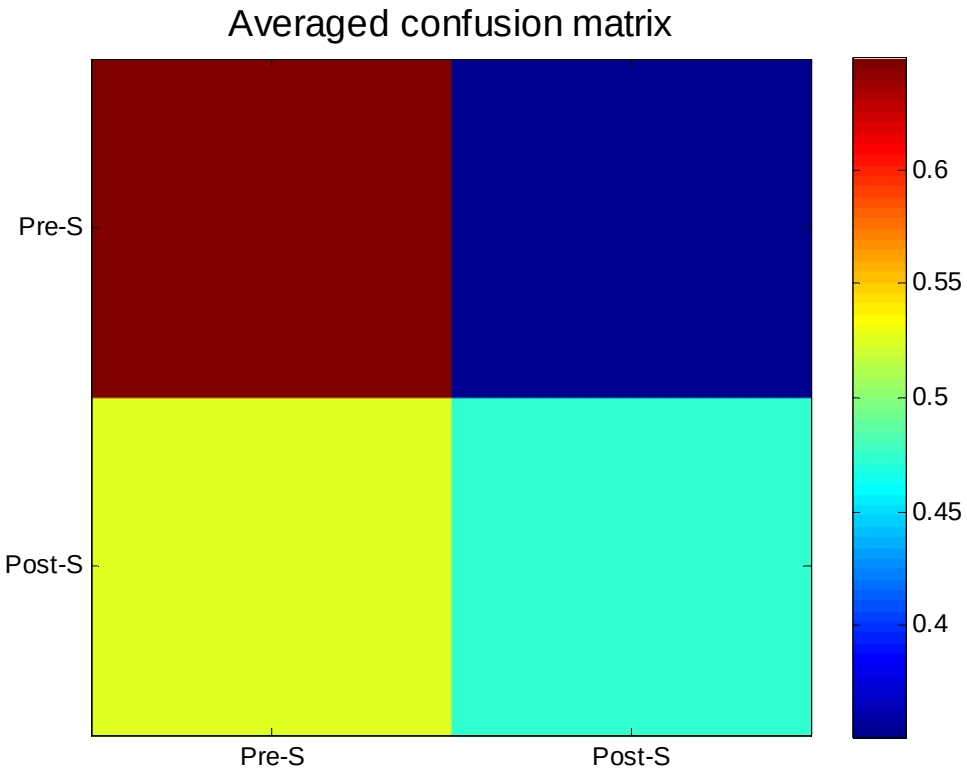


Table 4.12: Confusion Matrix of UHPLC-FTMS Urine Analysis Pre- and Post-Surgery

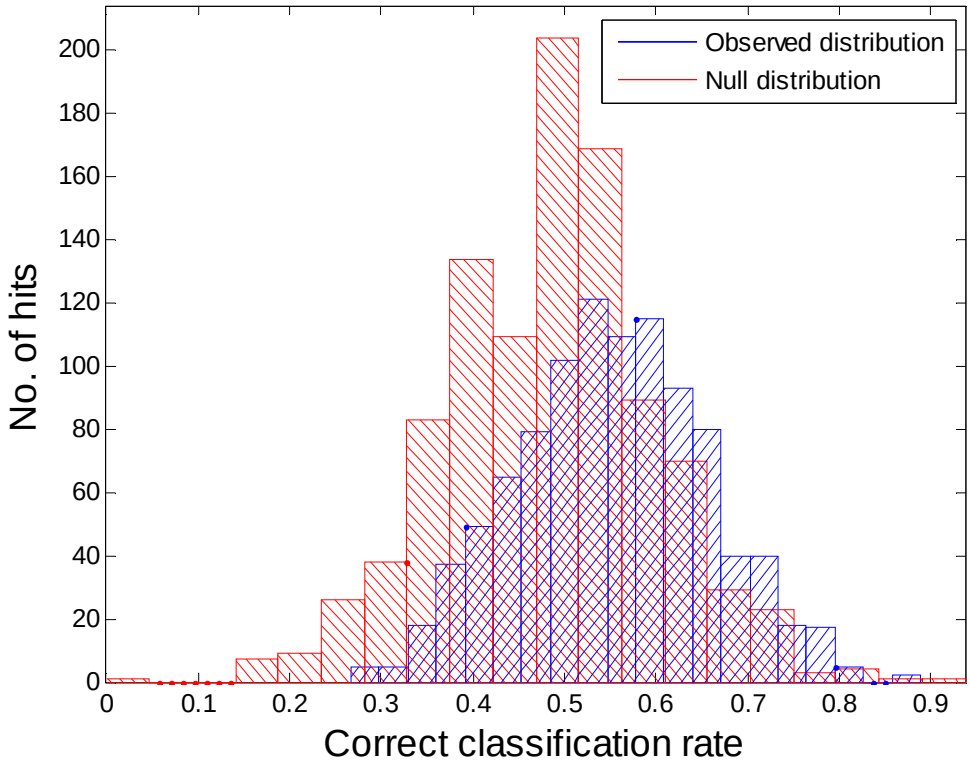
		Predicted	
		Pre Surgery	Post Surgery
Actual	Pre Surgery	64.9%	35.1%
	Post Surgery	52.4%	47.6%

Averaged CCR% = 55.2%

The averaged correct classification rate is 55.2%. It is possible to correctly predict the pre-surgery group in 64.9% of cases. However, it is only possible to predict the post-surgery group correctly in 47.6% of cases.

The bar chart shows that the observed distribution, of pre-surgery versus post-surgery, is not significantly different to the null distribution ($p>0.1$).

Figure 4.16: Bar Chart of UHPLC-FTMS Urine Analysis Pre- and Post-Surgery Observed and Null Distributions



p-value > 0.1

In this dataset, using urine samples from pre- and post-surgery IBD patients, analysed by UHPLC-FTMS, it is not possible to differentiate between pre-and post-surgery profiles.

4.4.6 Experiment 2 Summary

- There was no significant differentiation between the metabolomic profiles of pre- and post-surgery IBD patients when comparing either serum or urine samples on either UHPLC-FTMS (pos) or GC-ToF-MS platforms
- Serum analysis on the GC-ToF-MS platform had the greatest averaged correct classification rate (60.9% of cases)
- The post-surgical group in the serum analysis on the GC-ToF-MS platform has the highest true positive predictive rate (66.0%)
- The lowest rate of positive prediction was seen in serum analysis on the UHPLC-FTMS (pos) platform, with an averaged correct classification rate of only 39.7%

4.5 Experiment 3: Surgical IBD v HC

4.5.1 Experiment 3 Aims

In experiment 2 we were unable to differentiate between the metabolomic profiles of pre- and post-surgery IBD patients. In view of this we aimed to determine whether it is possible to differentiate between the metabolomic profiles of IBD patients who required surgical intervention, and healthy controls. Samples from the pre- and post-surgical groups were considered as one group in this experiment.

Analyses are carried out on serum and urine samples, and using GC-ToF-MS, and UHPLC-FTMS platforms.

Table 4.13 Experiment 3 number of samples analysed

	Surgical IBD	**Healthy Contols**
Serum samples	58	62
Urine samples	58	60

Confusion matrices and bar charts are used to visualise the results.

Bar charts are used to compare the null hypothesis, that there is no discerable difference between the profile of surgical IBD patients and healthy controls.

4.5.2 Experiment 3.1: Results

Figure 4.17: Visualisation of Confusion Matrix of GC-ToF-MS Serum Analysis Surgical IBD v HC

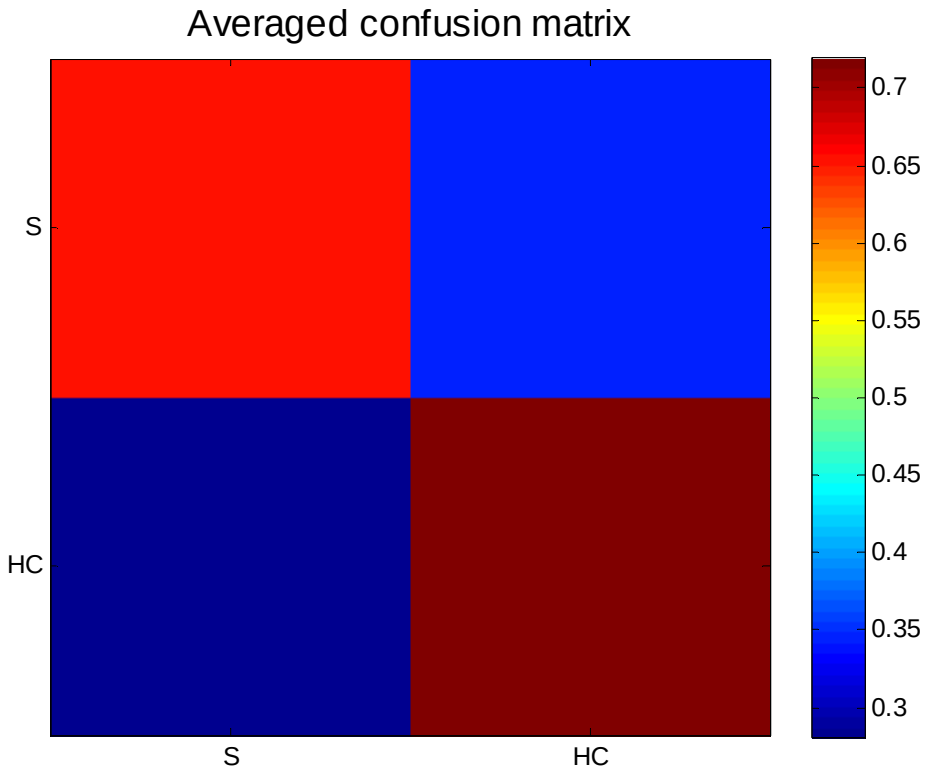


Table 4.14: Confusion Matrix of GC-ToF-MS Serum Analysis Surgical IBD v HC

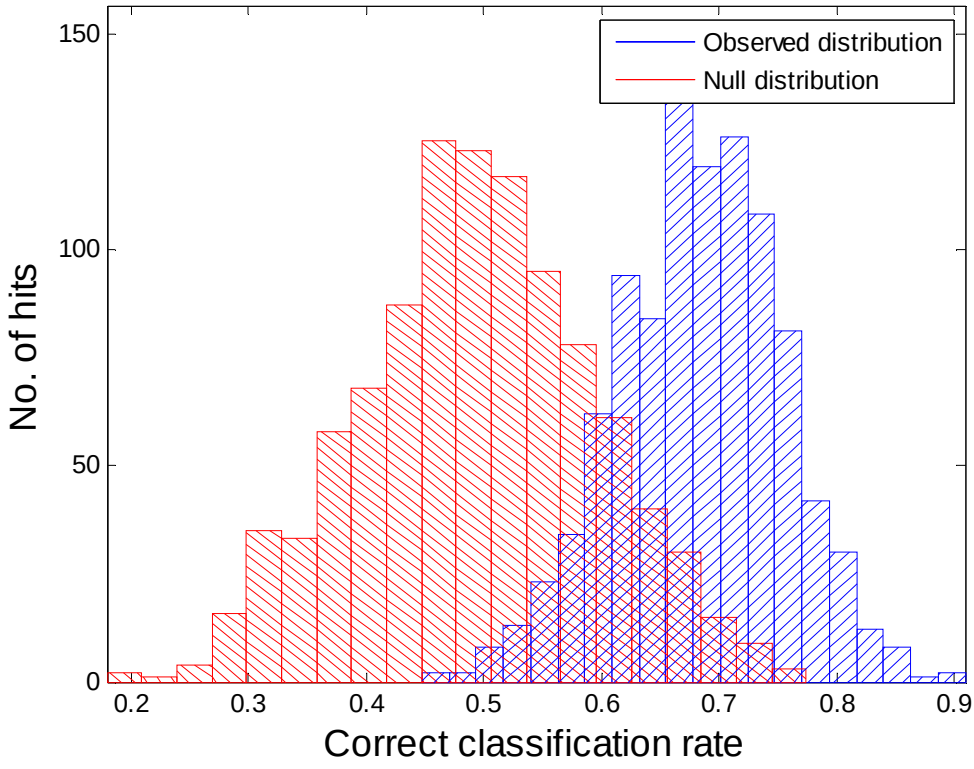
		Predicted	
		Surgical	HC
Actual	Surgical	65.6%	34.4%
	HC	28.1%	71.9%

Averaged CCR% = 68.3%

The averaged correct classification rate is 68.3%. It is possible to correctly predict the surgical IBD patients in 65.6% of cases, and in 71.9% of healthy controls.

The bar chart shows that the observed distribution, of surgical IBD patients versus healthy controls, is significantly different to the null distribution ($p<0.05$).

Figure 4.18: Bar Chart of GC-ToF-MS Serum Analysis Surgical IBD v HC Observed and Null Distributions



p-value < 0.05

In this dataset, using serum samples from surgical IBD patients and HCs, analysed by GC-ToF-MS, differentiation between the profiles is possible.

4.5.3 Experiment 3.2: Results

Figure 4.19: Visualisation of Confusion Matrix of UHPLC-FTMS Serum Analysis Surgical IBD v HC

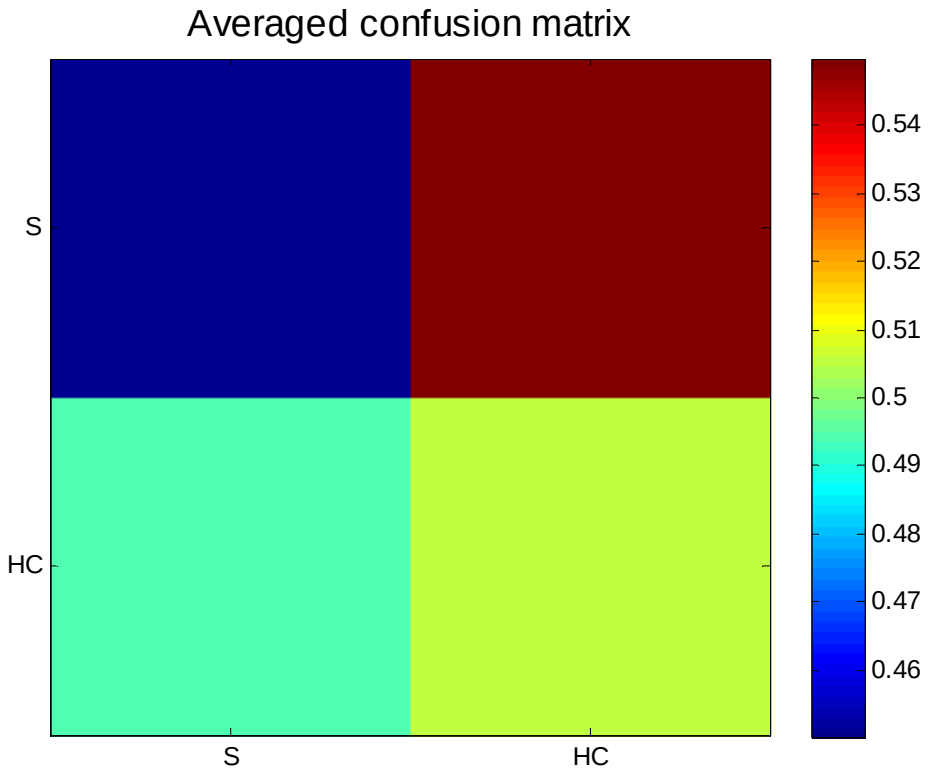


Table 4.15: Confusion Matrix of UHPLC-FTMS Serum Analysis Surgical IBD v HC

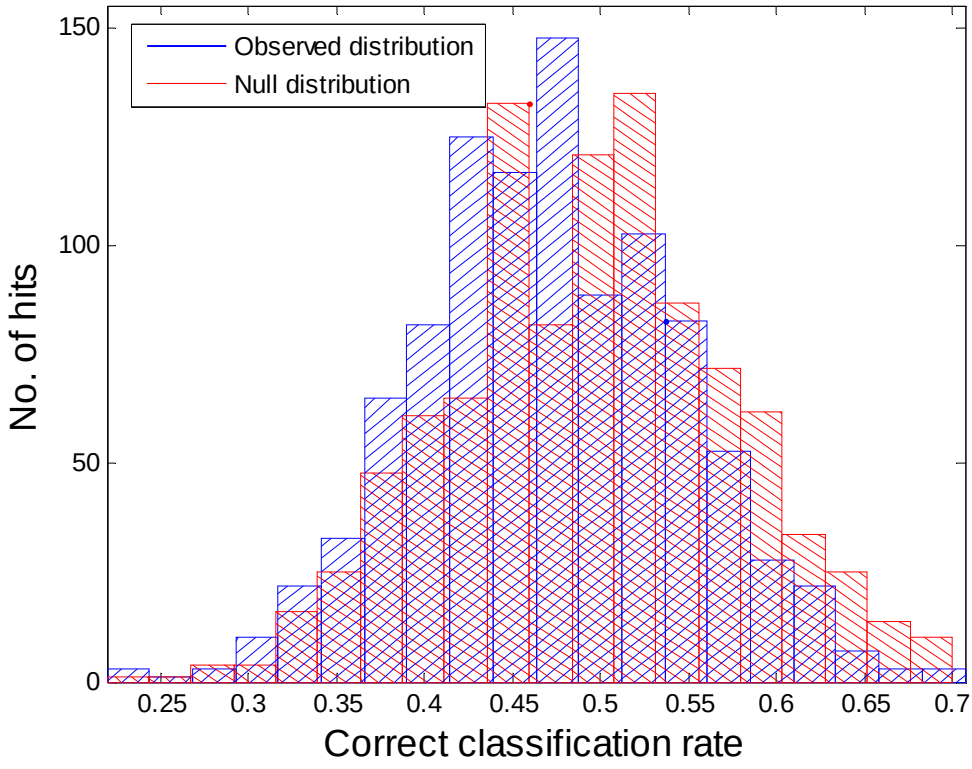
		Predicted	
		Surgical	HC
Actual	Surgical	45.0%	55.0%
	HC	49.5%	50.5%

Averaged CCR% = 47.2%

The averaged correct classification rate is 47.2%. It is only possible to correctly predict the surgical IBD group and the healthy controls in 45.0% and 50.5% of cases.

The bar chart shows that the observed distribution, of surgical IBD versus HCs, is not significantly different to the null distribution ($p>0.1$).

Figure 4.20: Bar Chart of UHPLC-FTMS Serum Analysis Surgical IBD v HC Observed and Null Distributions



p-value > 0.1

In this dataset, using serum samples from surgical IBD patients and HCs, analysed by UHPLC-FTMS, it is not possible to differentiate between the groups.

4.5.4 Experiment 3.3: Results

Figure 4.21: Visualisation of Confusion Matrix of GC-ToF-MS Urine Analysis Surgical IBD v HC

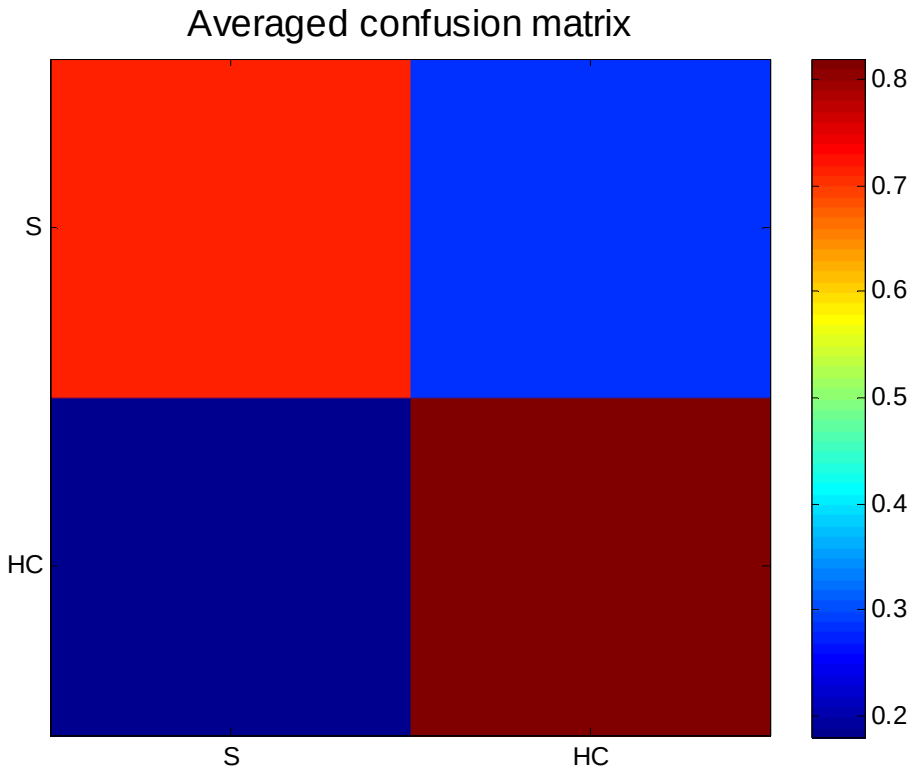


Table 4.16: Confusion Matrix of GC-ToF-MS Urine Analysis Surgical IBD v HC

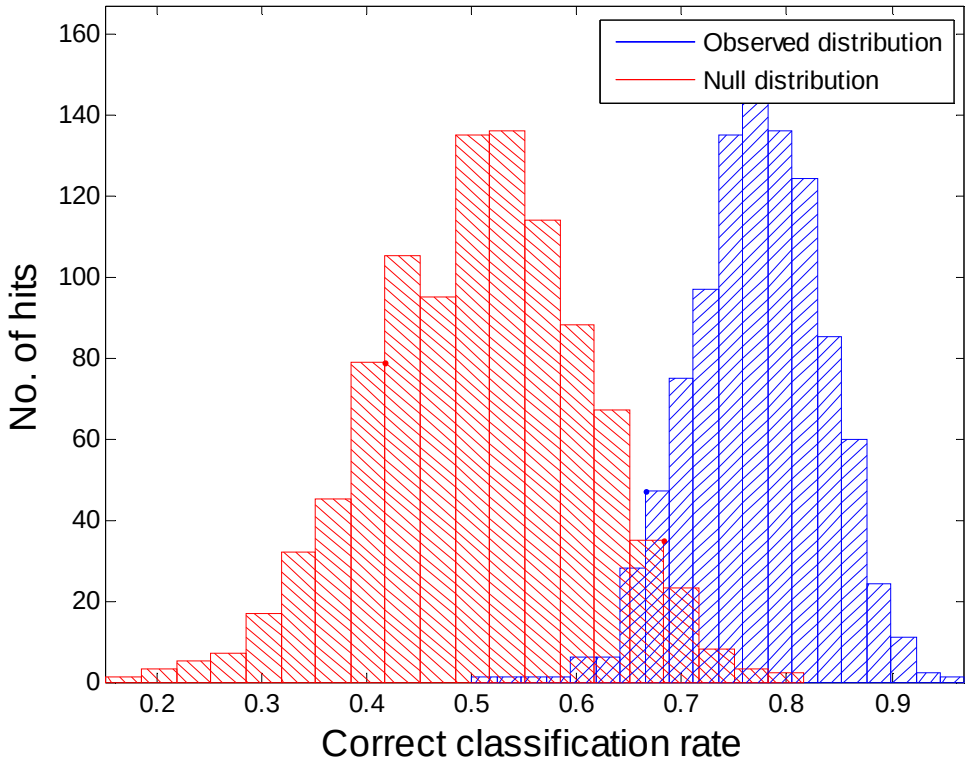
		Predicted	
		Surgical	HC
Actual	Surgical	71.9%	28.1%
	HC	18.0%	82.0%

Averaged CCR% = 77.2%

The averaged correct classification rate is 77.2%. It is possible to correctly predict the surgical IBD patients in 71.9% of cases, and in 82.0% of healthy controls.

The bar chart shows that the observed distribution, of surgical IBD patients versus healthy controls, is very significantly different to the null distribution ($p<0.01$).

Figure 4.22: Bar Chart of GC-ToF-MS Urine Analysis Surgical IBD v HC Observed and Null Distributions



p-value < 0.01

In this dataset, using urine samples from surgical IBD patients and HCs, analysed by GC-ToF-MS, differentiation between the profiles is very possible.

4.5.5 Experiment 3.4: Results

Figure 4.23: Visualisation of Confusion Matrix of UHPLC-FTMS Urine Analysis Surgical IBD v HC

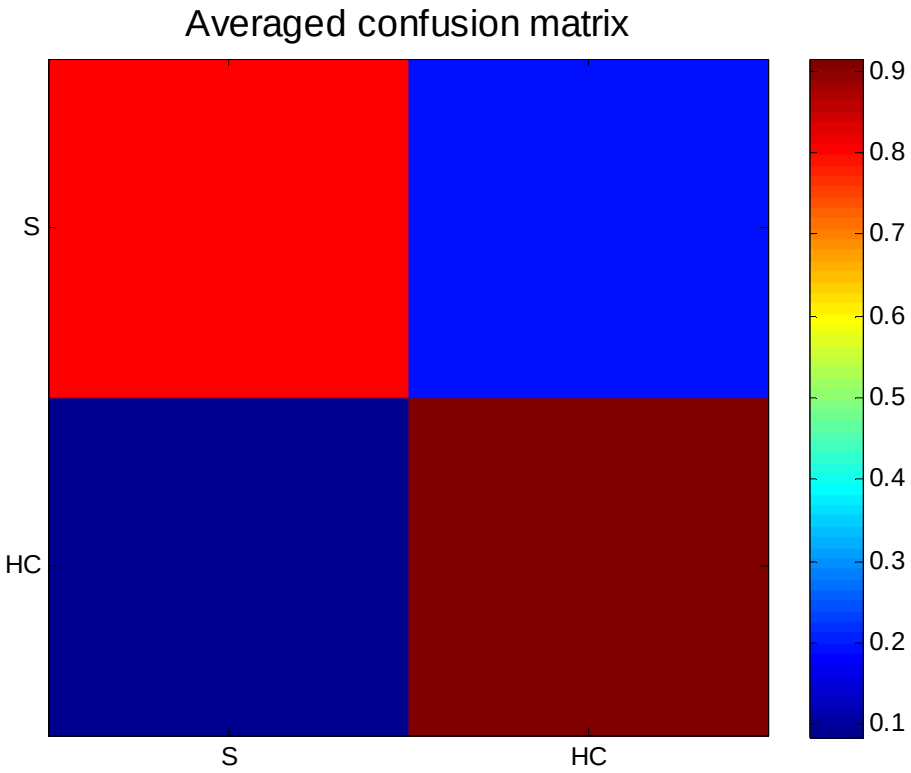


Table 4.17: Confusion Matrix of UHPLC-FTMS Urine Analysis Surgical IBD v HC

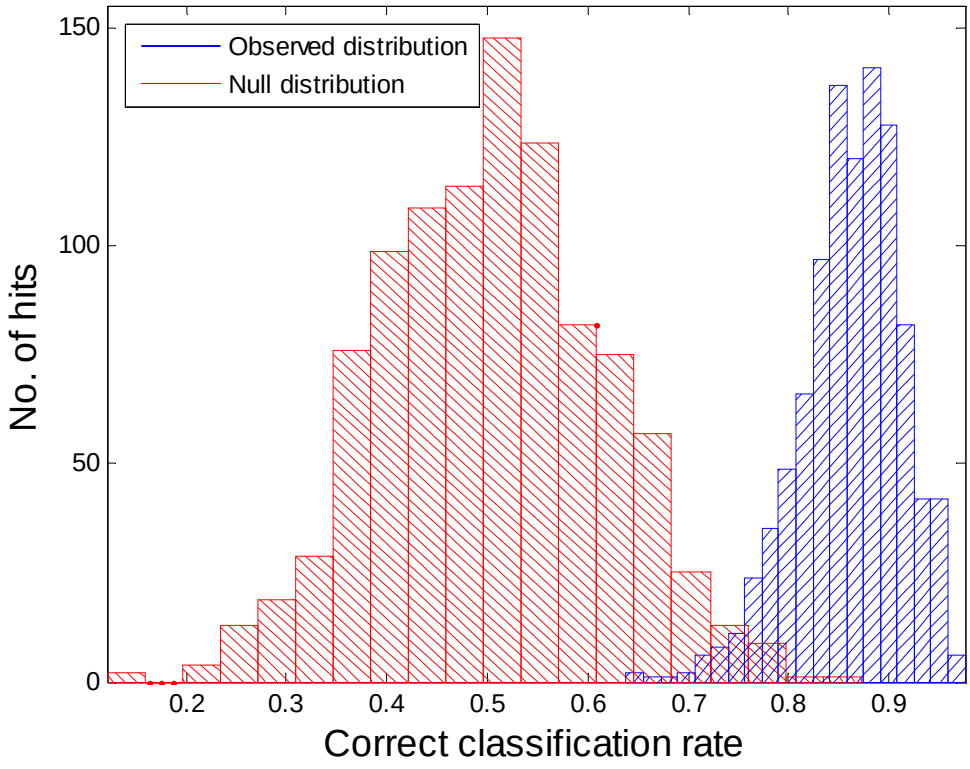
		Predicted	
		Surgical	HC
Actual	Surgical	80.1%	19.9%
	HC	8.5%	91.5%

Averaged CCR% = 86.2%

The averaged correct classification rate is 86.2%. It is possible to correctly predict the surgical IBD patients in 80.1% of cases, and in 91.5% of healthy controls.

The bar chart shows that the observed distribution, of surgical IBD patients versus healthy controls, is very significantly different to the null distribution ($p<0.001$).

Figure 4.24: Bar Chart of UHPLC-FTMS Urine Analysis Surgical IBD v HC Observed and Null Distributions



p-value < 0.001

In this dataset, using urine samples from surgical IBD patients and HCs, analysed by GC-ToF-MS, differentiation between the profiles is very possible.

4.5.6 Experiment 3 Summary

- Differentiation between the metabolomic profiles of surgical IBD patients and healthy controls is possible using serum and urine samples on both GC-ToF-MS and UHPLC-FTMS
- Urine samples show a greater level of significance of differentiation between surgical IBD patients than serum samples
- The most significant differentiation between surgical IBD samples and HCs is seen in urine samples analysed on the UHPLC-FTMS (pos) platform

4.6 Experiment 4: Pre- and Post- Biological Therapy

4.6.1 Experiment 4 Aims

To determine whether it is possible to differentiate between the metabolomic profiles of IBD patients immediately prior to commencing biological therapy for IBD (infliximab or adalimumab), and 2 weeks post initial treatment.

Analyses are carried out on serum and urine samples, and using GC-ToF-MS, and UHPLC-FTMS platforms.

Table 4.18 Experiment 4 number of samples analysed

	Pre-biological Therapy	**Post-biological Therapy**
Serum samples	24	24
Urine samples	24	23

Confusion matrices and bar charts are used to visualise the results.

Bar charts are used to compare the null hypothesis, that there is no discerable difference between the profile of pre and post biological therapy patients.

4.6.2 Experiment 4.1: Results

Figure 4.25: Visualisation of Confusion Matrix of GC-ToF-MS Serum Analysis Pre- and Post-Biological Therapy

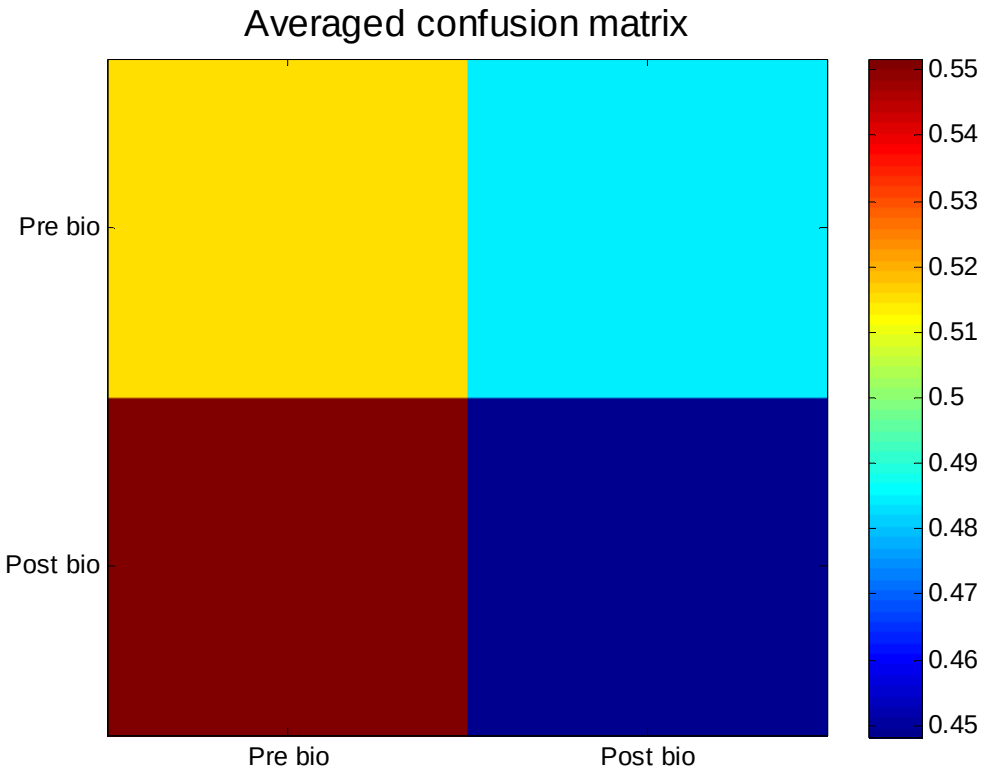


Table 4.19: Confusion Matrix of GC-ToF-MS Serum Analysis Pre- and Post-Biological Therapy

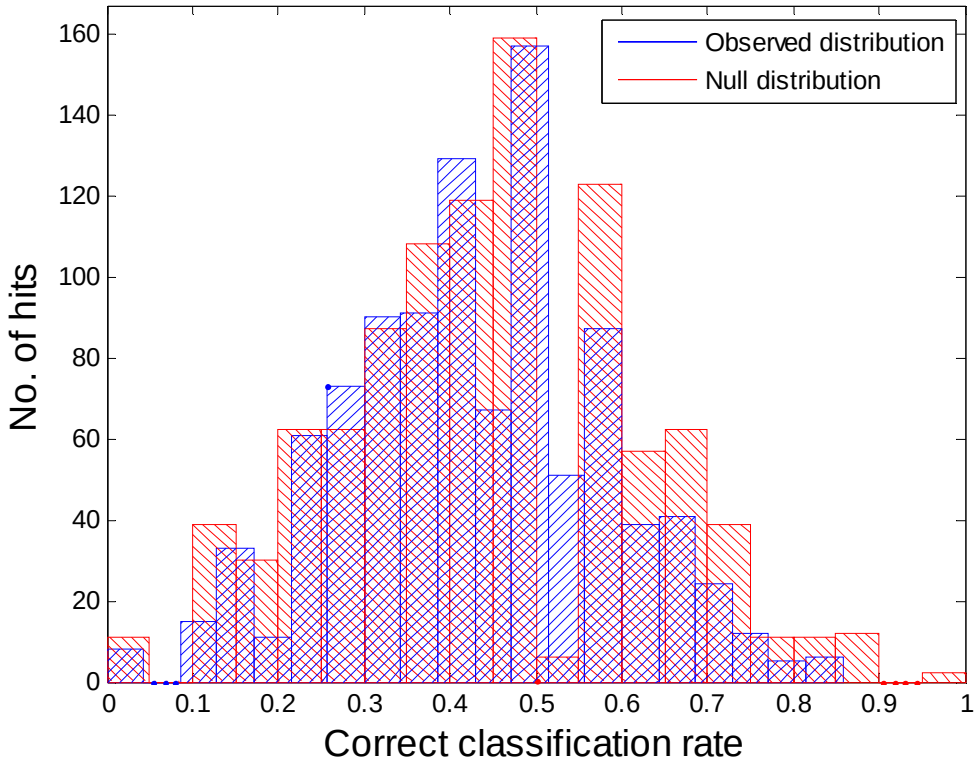
		Predicted	
		Pre biological	Post biological
Actual	Pre biological	51.5%	48.5%
	Post biological	55.2%	44.8%

Averaged CCR% = 43.7%

The averaged correct classification rate is 43.7%. It is possible to correctly predict the pre biological IBD patients in 51.5% of cases, but only in 44.8% of post biological patients.

The bar chart shows that the observed distribution, of pre-biological versus post-biological IBD patients, is not significantly different to the null distribution ($p>0.1$).

Figure 4.26: Bar Chart of GC-ToF-MS Serum Analysis Pre- and Post-Biological Therapy Observed and Null Distributions



p-value >0.1

In this dataset, using serum samples from pre- and post-biological IBD patients, analysed by GC-ToF-MS, it is not possible to differentiate between pre-and post-biological therapy profiles.

4.6.3 Experiment 4.2: Results

Figure 4.27: Visualisation of Confusion Matrix of UHPLC-FTMS Serum Analysis Pre- and Post-Biological Therapy

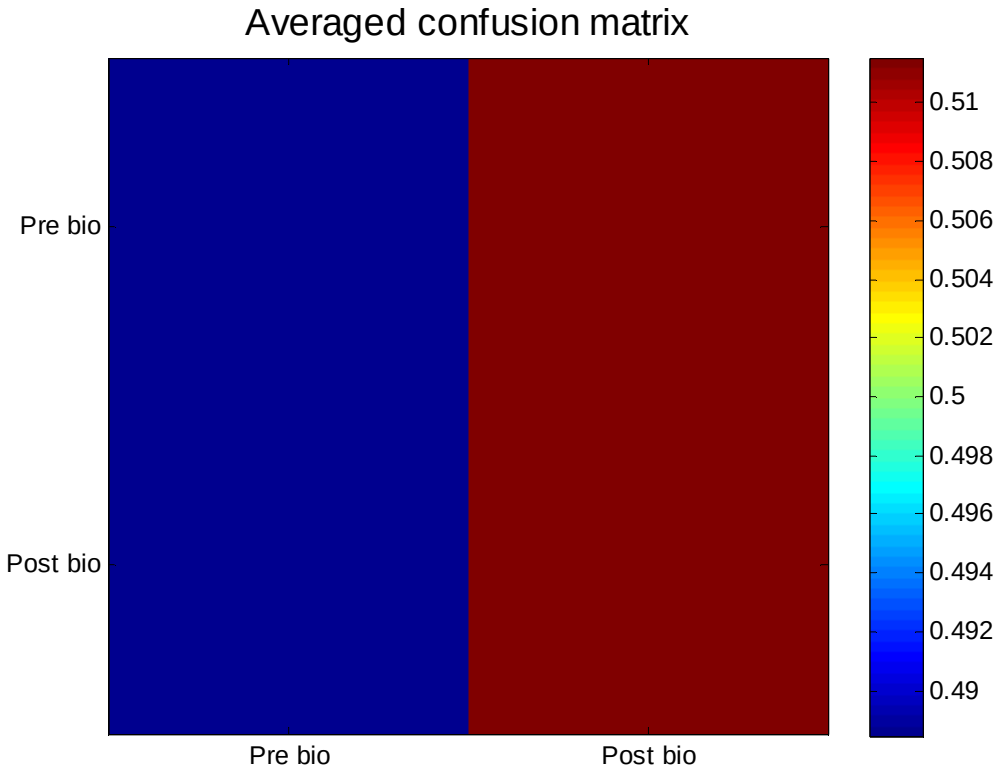


Table 4.20: Confusion Matrix of UHPLC-FTMS Serum Analysis Pre- and Post-Biological Therapy

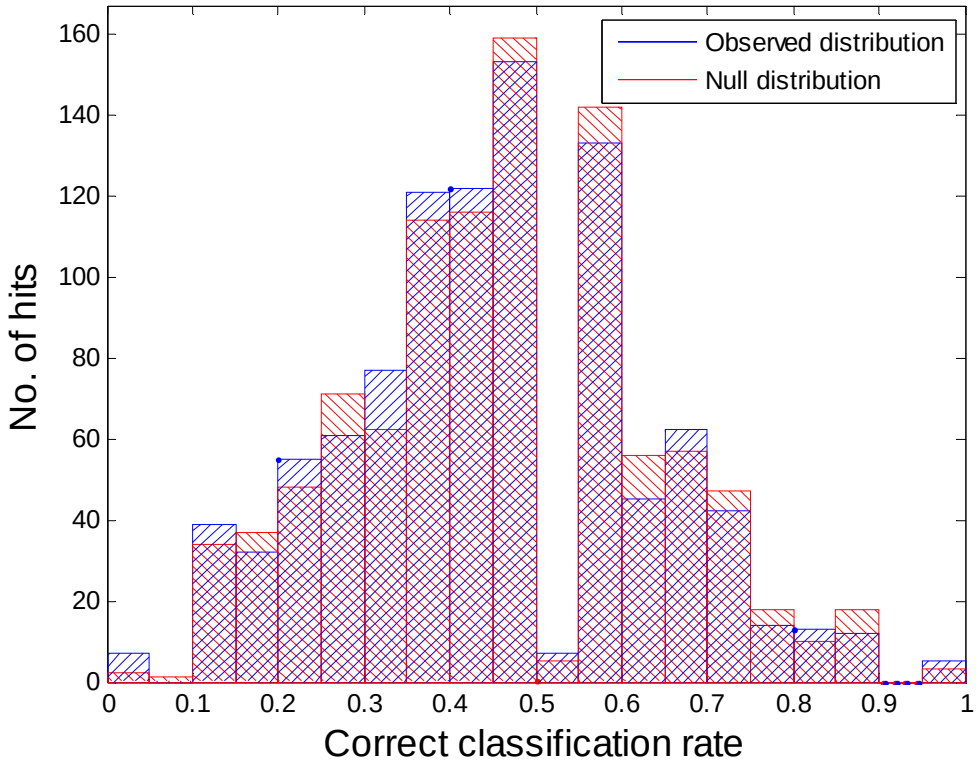
		Predicted	
		Pre biological	Post biological
Actual	Pre biological	48.9%	51.1%
	Post biological	48.9%	51.1%

Averaged CCR% = 46.1%

The averaged correct classification rate is 46.1%. It is possible to correctly predict the post biological IBD patients in 51.1% of cases, but only in 48.9% of pre biological patients.

The bar chart shows that the observed distribution, of pre-biological versus post-biological IBD patients, is not significantly different to the null distribution ($p>0.1$).

Figure 4.28: Bar Chart of UHPLC-FTMS Serum Analysis Pre- and Post-Biological Therapy Observed and Null Distributions



p-value > 0.1

In this dataset, using serum samples from pre- and post-biological IBD patients, analysed by UHPLC-FTMS (pos), it is not possible to differentiate between pre-and post-biological therapy profiles.

4.6.4 Experiment 4.3: Results

Figure 4.29: Visualisation of Confusion Matrix of GC-ToF-MS Urine Analysis Pre- and Post-Biological Therapy

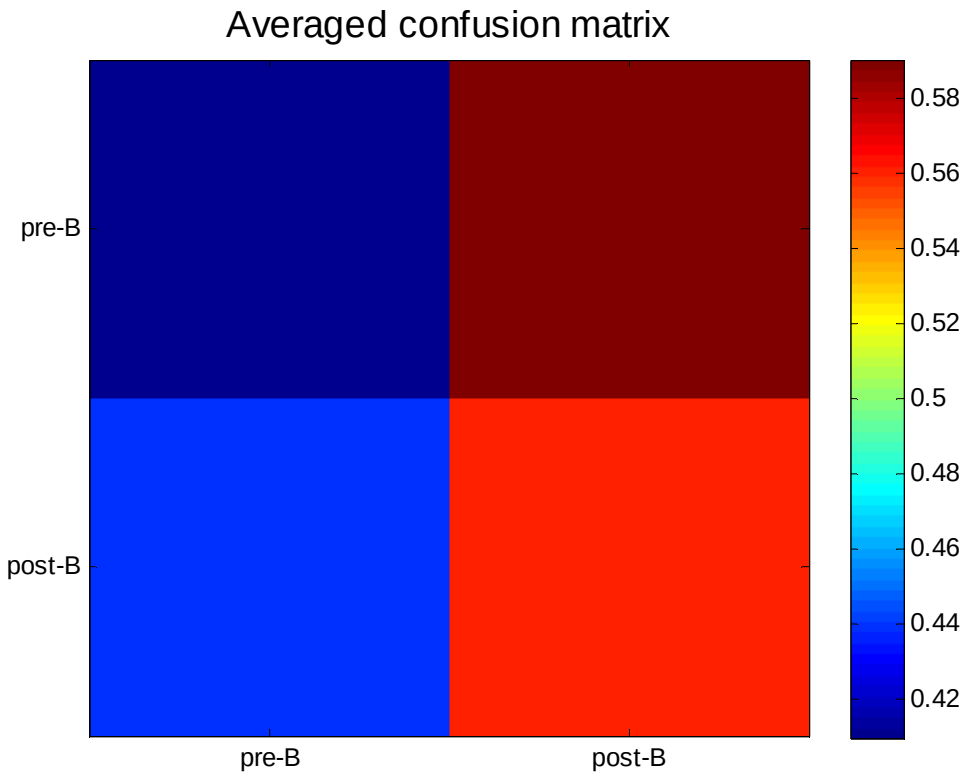


Table 4.21: Confusion Matrix of GC-ToF-MS Urine Analysis Pre- and Post-Biological Therapy

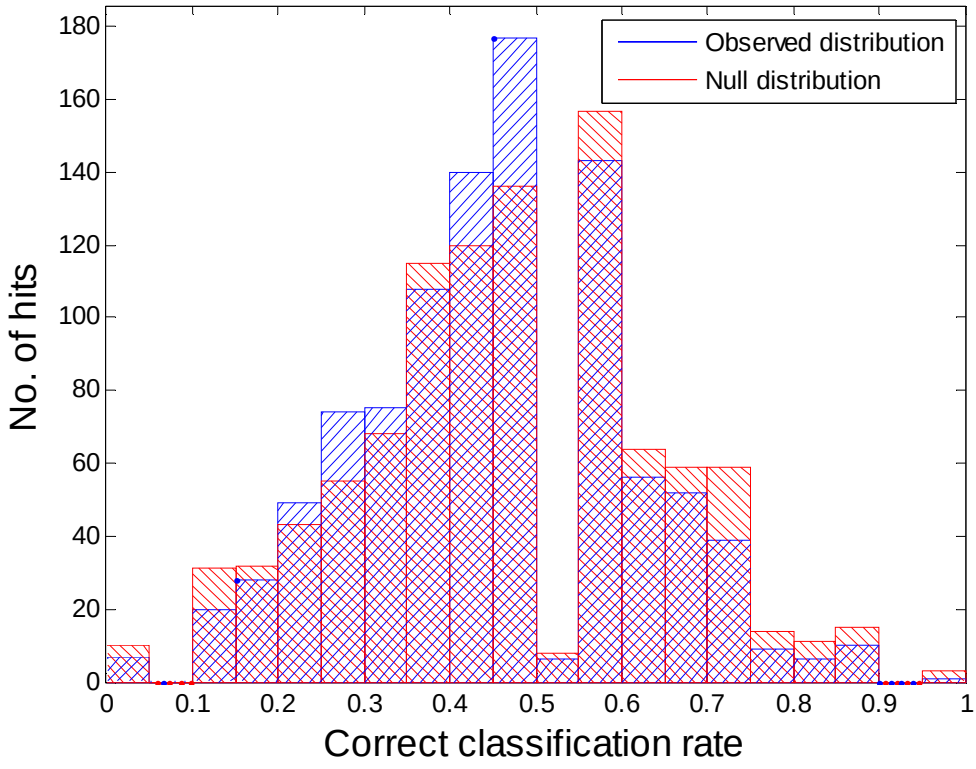
		Predicted	
		Pre biological	Post biological
Actual	Pre biological	41.0%	59.0%
	Post biological	44.0%	56.0%

Averaged CCR% = 46.1%

The averaged correct classification rate is 46.1%. It is possible to correctly predict the post biological IBD patients in 56.0% of cases, but only in 41.0% of pre biological patients.

The bar chart shows that the observed distribution, of pre-biological versus post-biological IBD patients, is not significantly different to the null distribution ($p>0.1$).

Figure 4.30: Bar Chart of GC-ToF-MS Urine Analysis Pre- and Post-Biological Therapy Observed and Null Distributions



p-value > 0.1

In this dataset, using urine samples from pre- and post-biological IBD patients, analysed by GC-ToF-MS, it is not possible to differentiate between pre-and post-biological therapy profiles.

4.6.5 Experiment 4.4: Results

Figure 4.31: Visualisation of Confusion Matrix of UHPLC-FTMS Urine Analysis Pre- and Post-Biological Therapy

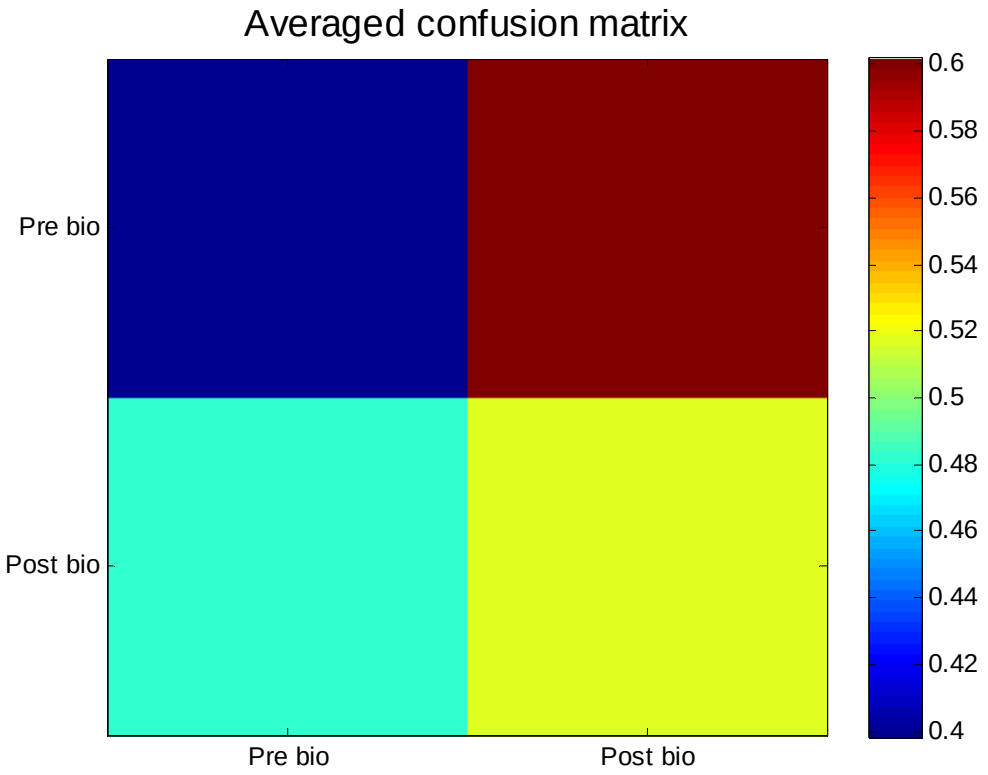


Table 4.22: Confusion Matrix of UHPLC-FTMS Urine Analysis Pre- and Post-Biological Therapy

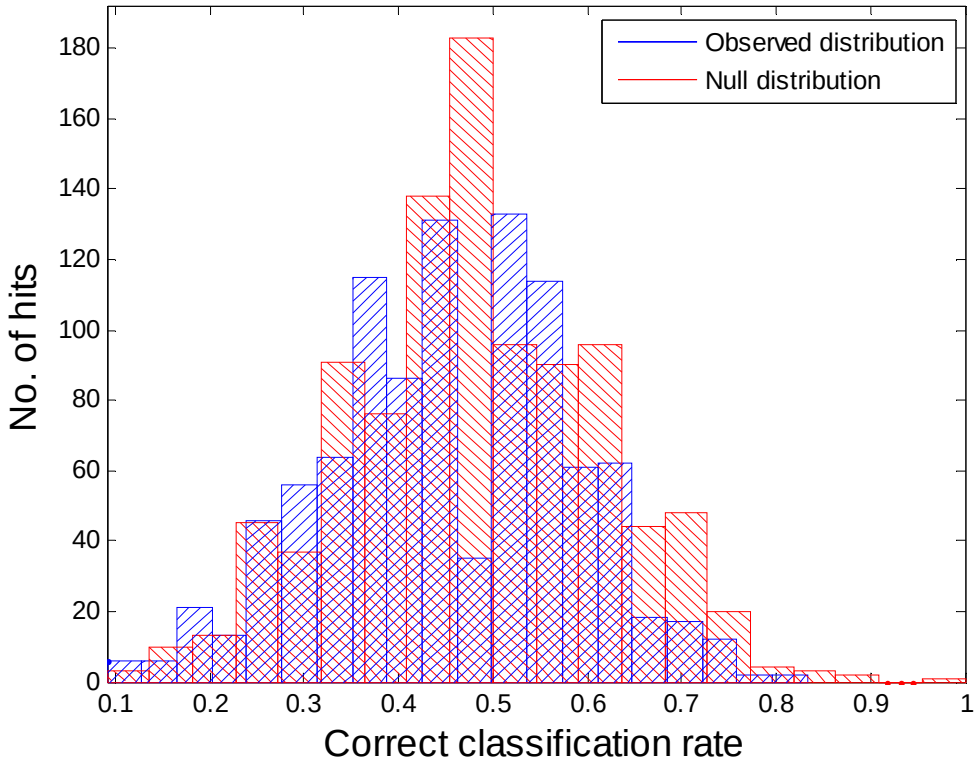
		Predicted	
		Pre biological	Post biological
Actual	Pre biological	43.4%	56.6%
	Post biological	37.4%	62.6%

Averaged CCR% = 45.2%

The averaged correct classification rate is 45.2%. It is possible to correctly predict the post biological IBD patients in 62.6% of cases, but only in 43.4% of pre biological patients.

The bar chart shows that the observed distribution, of pre-biological versus post-biological IBD patients, is not significantly different to the null distribution ($p>0.1$).

Figure 4.32: Bar Chart of UHPLC-FTMS Urine Analysis Pre- and Post-Biological Therapy Observed and Null Distributions



p-value > 0.1

In this dataset, using urine samples from pre- and post-biological IBD patients, analysed by UHPLC-FTMS (pos), it is not possible to differentiate between pre-and post-biological therapy profiles.

4.6.6 Experiment 4 Summary

- There was no significant differentiation between the metabolomic profiles of pre- and post-biological therapy IBD patients when comparing either serum or urine samples on either UHPLC-FTMS (pos) or GC-ToF-MS platforms
- UHPLC-FTMS (pos) Serum samples and GC-ToF-MS urine samples showed the highest average correct classification rate (46.1%)
- The lowest rate of positive prediction was seen in serum analysis on the GC-ToF-MS platform, with an averaged correct classification rate of only 43.7%
- Post-biological samples appear to have higher positive predictive rates than pre-biological samples

4.7 Experiment 5: Biological IBD v HC

4.7.1 Experiment 5 Aims

In experiment 4 we were unable to differentiate between the metabolomic profiles of pre- and post-biological therapy IBD patients. In view of this, we aimed to determine whether it is possible to differentiate between the metabolomic profiles of IBD patients who required biological therapy, and healthy controls. Samples from the pre- and post-biological groups were considered as one group in this experiment.

Analyses are carried out on serum and urine samples, and using GC-ToF-MS, and UHPLC-FTMS platforms.

Table 4.23 Experiment 5 number of samples analysed

	Biological IBD	**Healthy Controls**
Serum samples	48	62
Urine samples	47	60

Confusion matrices and bar charts are used to visualise the results.

Bar charts are used to compare the null hypothesis, that there is no discerable difference between the profile of surgical IBD patients and healthy controls.

4.7.2 Experiment 5.1: Results

Figure 4.33: Visualisation of Confusion Matrix of GC-ToF-MS Serum Analysis Biological Therapy IBD Patients v HC

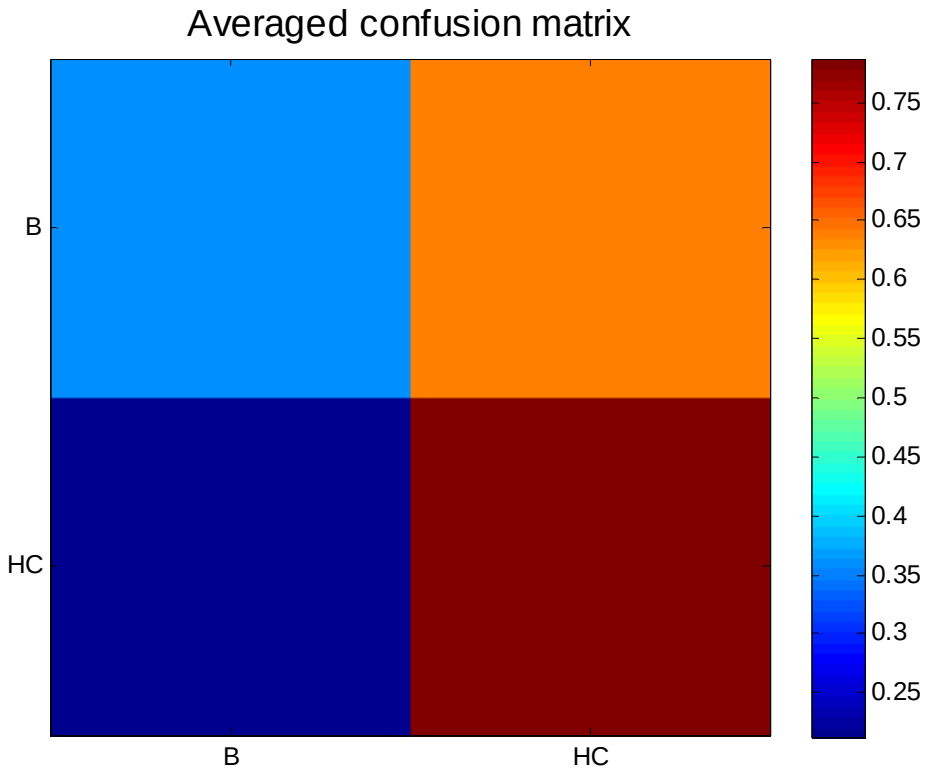


Table 4.24: Confusion Matrix of GC-ToF-MS Serum Analysis Biological Therapy IBD Patients v HC

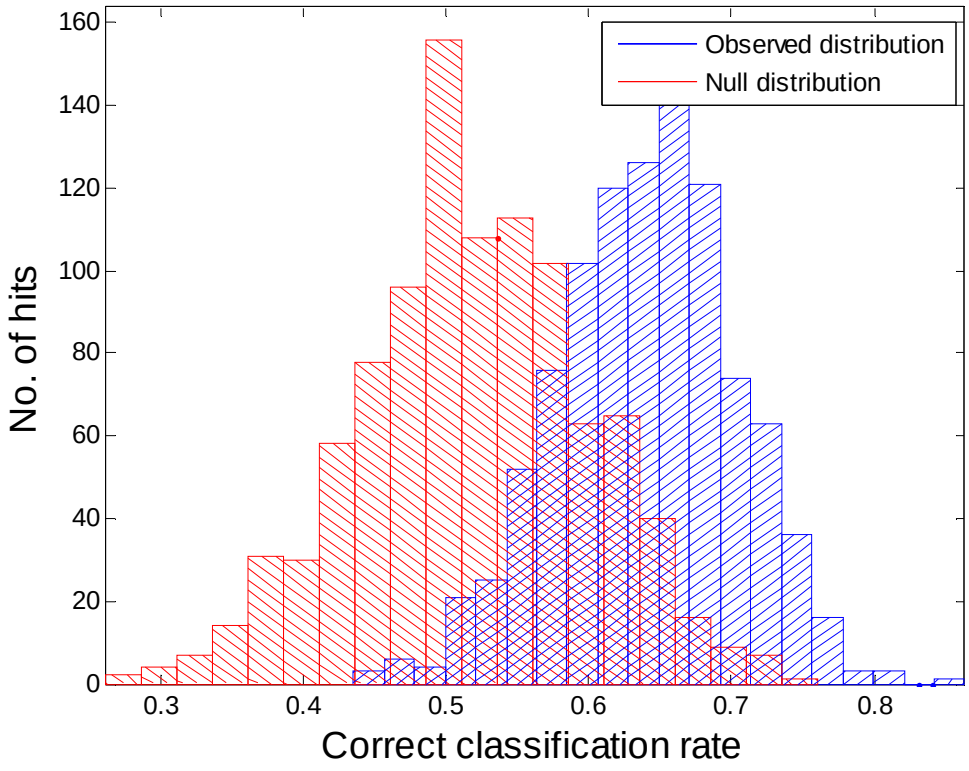
		Predicted	
		Biological IBD	HC
Actual	Biological IBD	35.8%	64.2%
	HC	21.3%	78.7%

Averaged CCR% = 64.8%

The averaged correct classification rate is 64.8%. It is possible to correctly predict the biological IBD patients in only 35.8% of cases, however, in 78.7% of cases HC are correctly identified as HC.

The bar chart shows that the observed distribution, of biological IBD versus HC, is not significantly different to the null distribution ($p>0.1$).

Figure 4.34: Bar Chart of GC-ToF-MS Serum Analysis Biological Therapy IBD Patients v HC Observed and Null Distributions



p-value > 0.1

In this dataset, using serum samples from biological IBD patients and healthy controls analysed by GC-ToF-MS, it is not possible to differentiate between the profiles of IBD patients requiring biological therapy and healthy controls.

4.7.3 Experiment 5.2: Results

Figure 4.35: Visualisation of Confusion Matrix of UHPLC-FTMS Serum Analysis Biological Therapy IBD Patients v HC

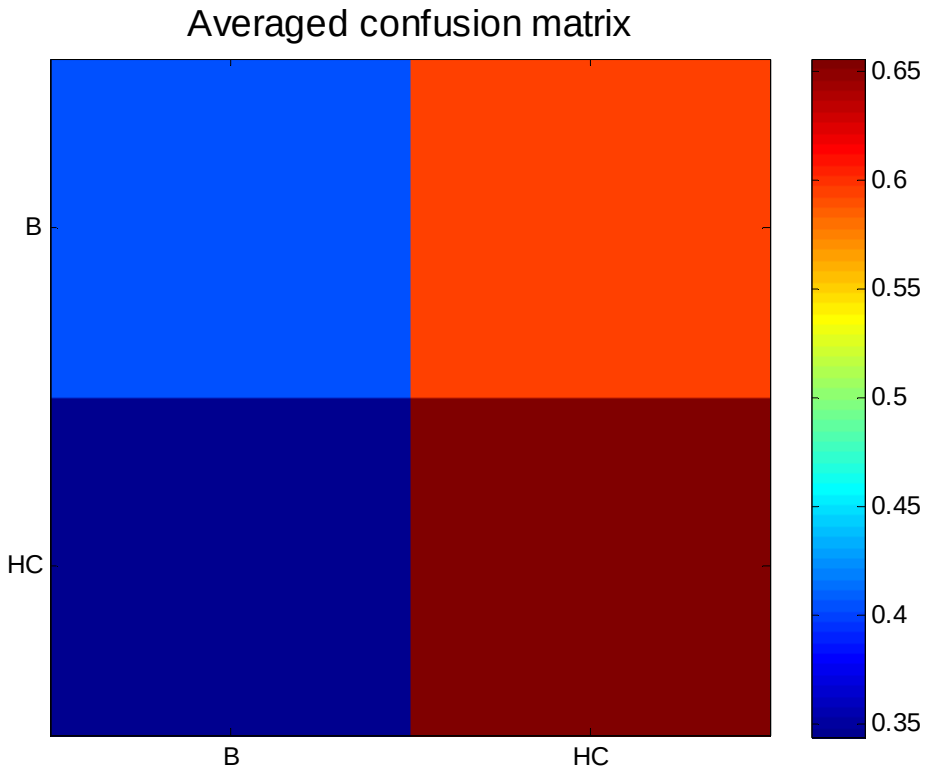


Table 4.25: Confusion Matrix of UHPLC-FTMS Serum Analysis Biological Therapy IBD Patients v HC

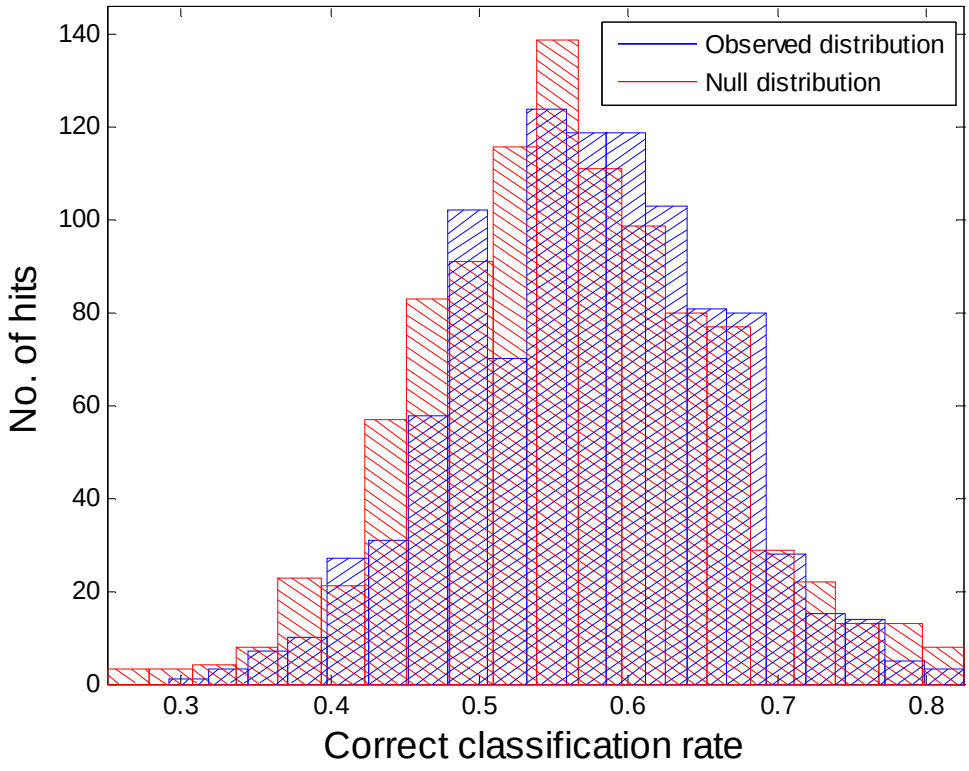
		Predicted	
		Biological IBD	HC
Actual	Biological IBD	40.4%	59.6%
	HC	34.4%	65.6%

Averaged CCR% = 57.1%

The averaged correct classification rate is 57.1%. It is possible to correctly predict the biological IBD patients in only 40.4% of cases, however, in 65.5% of cases HC are correctly identified as HC.

The bar chart shows that the observed distribution, of biological IBD versus HC, is not significantly different to the null distribution ($p>0.1$).

Figure 4.36: Bar Chart of UHPLC-FTMS Serum Analysis Biological Therapy IBD Patients v HC Observed and Null Distributions



p-value > 0.1

In this dataset, using serum samples from biological IBD patients and healthy controls analysed by UHPLC-FTMS (pos), it is not possible to differentiate between the profiles of IBD patients requiring biological therapy and healthy controls.

4.7.4 Experiment 5.3: Results

Figure 4.37: Visualisation of Confusion Matrix of GC-ToF-MS Urine Analysis Biological Therapy IBD Patients v HC

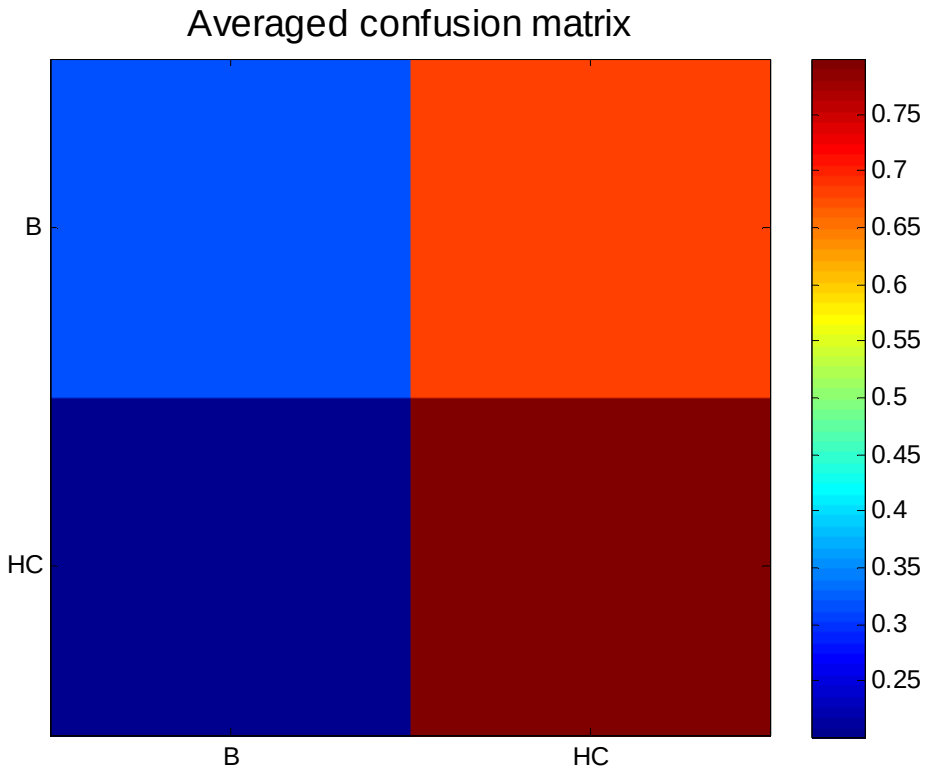


Table 4.26: Confusion Matrix of GC-ToF-MS Urine Analysis Biological Therapy IBD Patients v HC

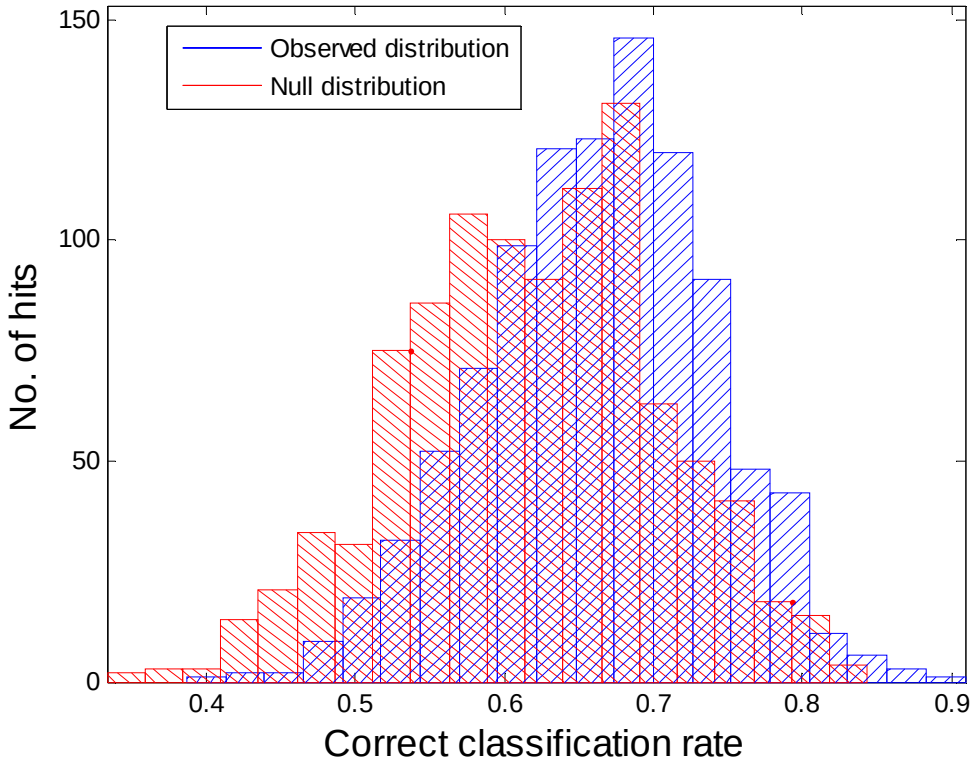
		Predicted	
		Biological IBD	HC
Actual	Biological IBD	31.8%	68.2%
	HC	20.1%	79.9%

Averaged CCR% = 66.4%

The averaged correct classification rate is 66.4%. It is possible to correctly predict the biological IBD patients in only 31.8% of cases, however, in 79.9% of cases HC are correctly identified as HC.

The bar chart shows that the observed distribution, of biological IBD versus HC, is not significantly different to the null distribution ($p>0.1$).

Figure 4.38: Bar Chart of GC-ToF-MS Urine Analysis Biological Therapy IBD Patients v HC Observed and Null Distributions



p-value > 0.1

In this dataset, using urine samples from biological IBD patients and healthy controls analysed by GC-ToF-MS, it is not possible to differentiate between the profiles of IBD patients requiring biological therapy and healthy controls.

4.7.5 Experiment 5.4: Results

Figure 4.39: Visualisation of Confusion Matrix of UHPLC-FTMS Urine Analysis Biological Therapy IBD Patients v HC

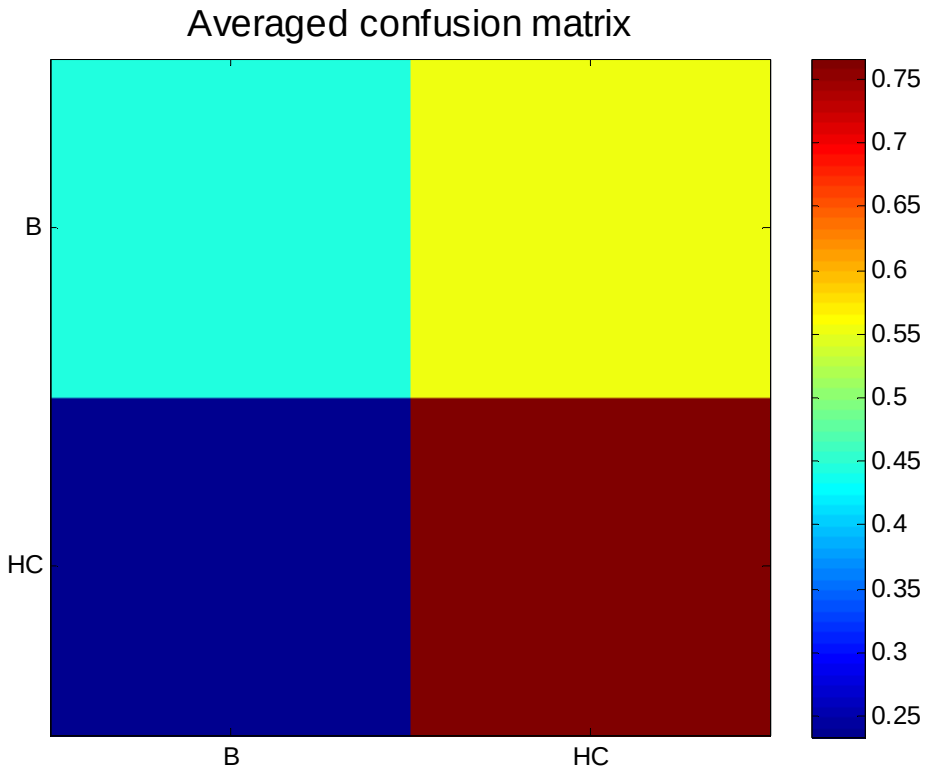


Table 4.27: Confusion Matrix of UHPLC-FTMS Urine Analysis Biological Therapy IBD Patients v HC

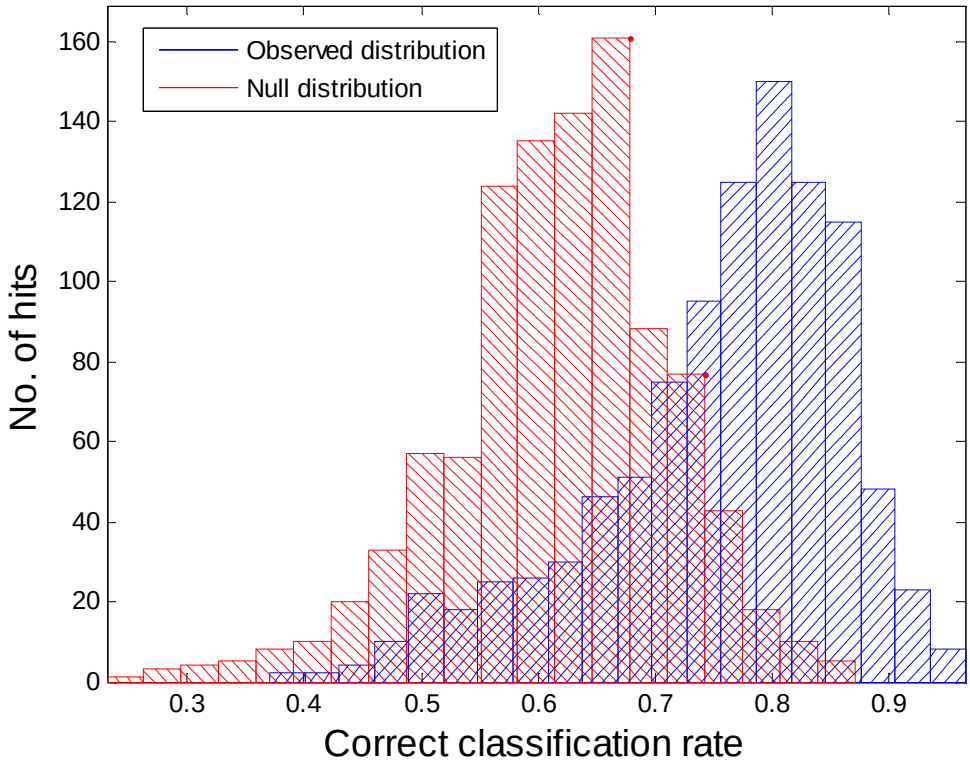
		Predicted	
		Biological IBD	HC
Actual	Biological IBD	43.8%	56.2%
	HC	11.5%	88.5%

Averaged CCR% = 75.8%

The averaged correct classification rate is 75.8%. It is possible to correctly predict the biological IBD patients in 43.8% of cases, however, in 88.5% of cases HC are correctly identified as HC.

The bar chart shows that the observed distribution, of biological IBD versus HC, is not significantly different to the null distribution (0.1 > p-value > 0.05).

Figure 4.40: Bar Chart of UHPLC-FTMS Urine Analysis Biological Therapy IBD Patients v HC Observed and Null Distributions



0.1 > p-value > 0.05

In this dataset, using urine samples from biological IBD patients and healthy controls analysed by GC-ToF-MS, it is not possible to differentiate between the profiles of IBD patients requiring biological therapy and healthy controls.

4.7.6 Experiment 5 Summary

- It is not possible to differentiate between the metabolomic profiles of biological IBD patients and healthy controls using either serum and urine samples on either GC-ToF-MS or UHPLC-FTMS platforms
- Urine samples analysed on the UHPLC-FTMS (pos) platform show the highest averaged correct classification rate (75.8%)
- Urine samples analysed on the UHPLC-FTMS (pos) platform show HC to have the highest postive predictability (88.5%)
- Serum samples analysed on the UHPLC-FTMS (pos) platform have the lowest averaged correct classification rate (57.1%)

4.8 Metabolite Identification

4.8.1 Important Metabolites

For each of the groups studied significant metabolites were identified for both urine and serum samples, and using both the UHPLC-FTMS and GC-ToF-MS platforms. In each of the experiments, the differentiating variables (metabolites) between the groups being interrogated are studied. As previously discussed, classification of metabolites is carried out using their mass spectral features, and identification using the Human Metabolome Database (www.hmdb.ca) for UHPLC-FTMS samples, and with in-house library spectra with the help of retention indices as well as the NIST libraries for GC-ToF-MS samples.

Using KEGG (Kyoto Encyclopedia of Genes and Genomes) (www.kegg.jp), PubMed (http://www.ncbi.nlm.nih.gov/pubmed), PubChem (https://pubchem.ncbi.nlm.nih.gov/), DrugBank (www.drugbank.ca), and LIPID MAPS (Lipid Metabolites and Pathways Strategy) (www.lipidmaps.org), metabolites were considered with regards to their biological relevance in IBD.

Box plots are used to visualise the differences seen in statistically significant metabolites after Bonferroni correction for group in each experiment.

In paired samples, the differences seen between pre and post intervention samples in an important variable are visualised using bar charts.

A full list of identified metabolites is attached as appendix 8.4.

4.8.2 Experiment 6: Metabolite Identification UC v CD v HC

In this experiment we aim to identify metabolites that differentiate between UC, CD and healthy controls.

For each of the variables identified below a search of the mass spectra relating to the specific mass / charge ratio is carried out. The ten metabolites with the smallest Delta (i.e. the ten metabolites which are closest to the exact m/z figure), are identified and each one investigated to determine biological relevance in the clinical setting described (in this experiment considering the differences between UC, CD and HC).

4.8.3 Experiment 6.1: Metabolite Identification UC v CD v HC UHPLC-FTMS

Table 4.28: Experiment 6 number of samples analysed

	Heathly Controls	**UC**	**CD**
Serum samples	62	127	128
Urine samples	60	125	127

Table 4.29: Important Variables Identified UC v CD v HC UHPLC-FTMS

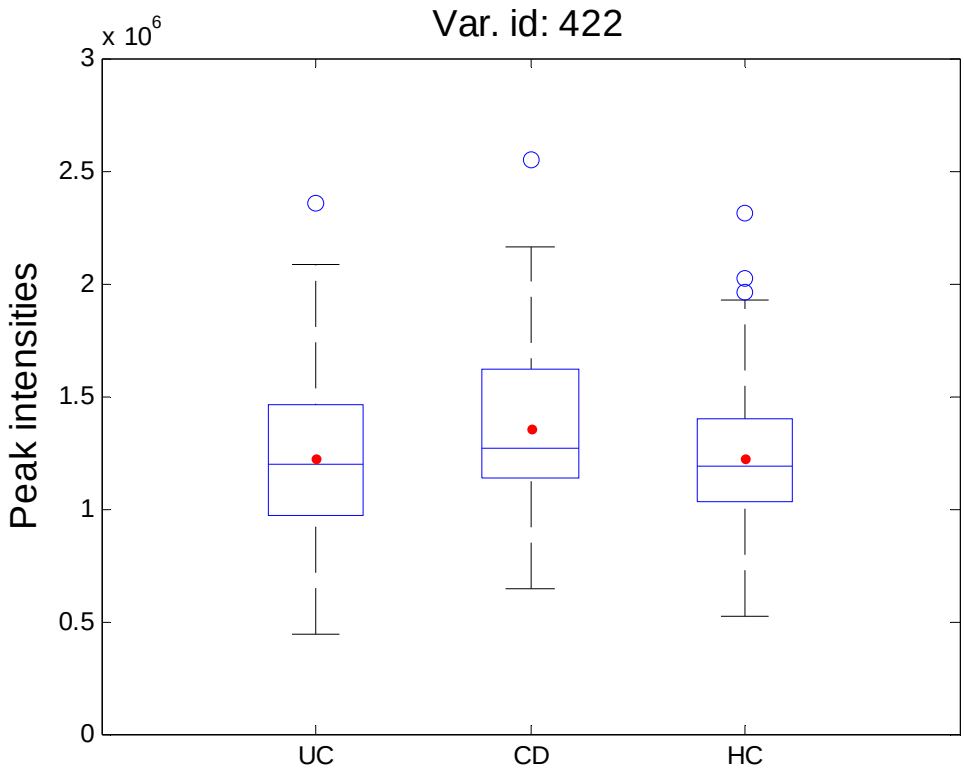
Variable ID	**Biofluid**	**m/z**	**Retention time**	**p value**	**q value**
422	Serum	337.1867552	816.16755	0.00321	0.03531
12	Urine	106.0362854	240.0221	7.96E-05	0.0066068
148	Urine	149.011087	1321.0958	0.00042576	0.03533808
562	Urine	243.1580154	502.09675	4.20E-05	0.003486
727	Urine	296.1481922	181.38205	0.00033741	0.02800503
743	Urine	302.1587031	143.3935	2.38E-05	0.0019754
800	Urine	334.0909909	299.601	1.03E-05	0.0008549

Table 4.29.1: Mass spectra search for 337.1867552 m/z

Compound	**Name**	**Adduct**	**Adduct MW (Da)**	**Compound MW (Da)**	**Delta**	**Class**
HMDB01431	Pyridoxamine	2M+H	337.187031	168.089878	0.0002758	Pyridines and derivatives
HMDB30263	Rhazidigenine Nb-oxide	M+Na	337.188646	314.199428	0.0018908	Indoles and derivatives
HMDB40682	23-trans-p-Coumaroyl oxytormentic acid	M+H+Na	337.189181	650.381869	0.0024258	Prenol lipids
HMDB40650	3-trans-Caffeoyltormentic acid	M+H+Na	337.189181	650.381869	0.0024258	Prenol lipids
HMDB35966	Momordicin II	M+H+K	337.189259	634.408083	0.0025038	Steroids and steroid

						derivatives
HMDB29621	Lucyoside Q	M+H+K	337.189259	634.408083	0.0025038	Prenol lipids
HMDB39329	Soyasapogenol B 3-O-b-D-glucuronide	M+H+K	337.189259	634.408083	0.0025038	Not classified
HMDB38452	28-Glucosylpomolate	M+H+K	337.189259	634.408083	0.0025038	Prenol lipids
HMDB35917	Momordicoside L	M+H+K	337.189259	634.408083	0.0025038	Prenol lipids
HMDB29606	Maslinic acid 3-O-b-D-glucoside	M+H+K	337.189259	634.408083	0.0025038	Not classified

Figure 4.41: Boxplot Serum Variable ID 422 UC v CD v HC UHPLC-FTMS



p=0.00321

In this experiment, serum peak intensities of variable ID 422 are higher in CD than in UC or HCs. The metabolites pyridoxamine and soyasapogenol B 3-O-b-D-glucuronide have been identified as potentially biologically relevant.

Pyridoxamine

Pyridoxamine is the 4-aminomethyl form of vitamin B6. The biologically active form of vitamin B6 is pyridoxal-5-phosphate (PLP). Whilst a relationship between vitamin B6 and inflammation has been postulated, it is yet to be defined. Plasma PLP concentrations have been shown to be reduced in rheumatoid arthritis patients, inversely related to the severity of disease (Roubenoff, Roubenoff et al. 1995). Saibeni et al (Saibeni, Cattaneo et al. 2003) report that plasma PLP concentrations are significantly lower in IBD patients than in healthy controls, and that the prevalence of low PLP (<20nmol/L) was significantly higher amongst patients with active disease compared to quiescent disease (26.9% v 2.9% ; $p \leq 0.001$). Selhub et al (Selhub, Byun et al. 2013) have shown that, in a rodent model, both low and high plasma PLP concentrations significantly reduce histological and molecular features of colonic inflammation. The findings of deficiency are in keeping with previous work (Benight, Stoll et al. 2011), however, this study raises the possibility of vitamin B6 modulation as a therapy in IBD.

Soyasapogenol B 3-O-b-D-glucuronide

Soyasapogenols, aglycones of soyasaponins, do not occur in soyabean naturally but can be formed by acid or alkaline hydrolysis of soyasaponins and therefore may exist in processed soy products. Soyasaponins and soyasapogenols have been reported to possess anti-inflammatory, anticarcinogen, antiviral and antioxidant activities, as well as hepatoprotective and cardiovascular protective effects. Soyasaponins may inhibit NF-κB activation thus exerting anti-inflammatory effects, however, soyasapogenols exhibit no inhibitory effect on the production of NO, iNOS, and TNF-α, or on the iNOS enzyme activity in LPS-stimulated macrophages, indicating that sugar chains in the structures of soyasaponins are critical to their anti-inflammatory activities (Guang, Chen et al. 2014).

In 2,4,6-trinitrobenzenesulfonic acid (TNBS)- induced colitic mice, orally administered soyasaponins Ab and I potently ameliorated body weight reduction, colon shortening, macroscopic score, and myeloperoxidase activity, the expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), and activation of the transcription factor nuclear factor-κB (NF-κB) (Lee, Park et al. 2011, Lee, Park et al. 2010). Soyasaponin I suppresses the expression of PGE2 and IL-6, and also suppresses the activation of NF-κB, reducing the levels of the lipid peroxides malondialdehyde (MDA) and 4-hydroxy-2-nonenal and increasing the glutathione content as well as the superoxide dismutase (SOD) and catalase activities (Lee, Park et al. 2010). This suggests that soyasaponin I may ameliorate colitis by inhibiting NF-κB activation and consequently diminishing its ability to scavenge the lipid peroxides produced by TNBS. Soyasaponin Ab also inhibits the expression of TLR4 and the phosphorylation of IRAK1, the inhibitor of NF-κB kinase (IKK)-β, and the NF-κB subunit p65 in the colon of TNBS- induced colitic mice (Lee, Park et al. 2011).

Soyasaponin I has also been shown to have moderate activities against Escherichia coli and Candida albicans, and soysapogenol A has shown moderate anti-HSV-1 activity (Guang, Chen et al. 2014).

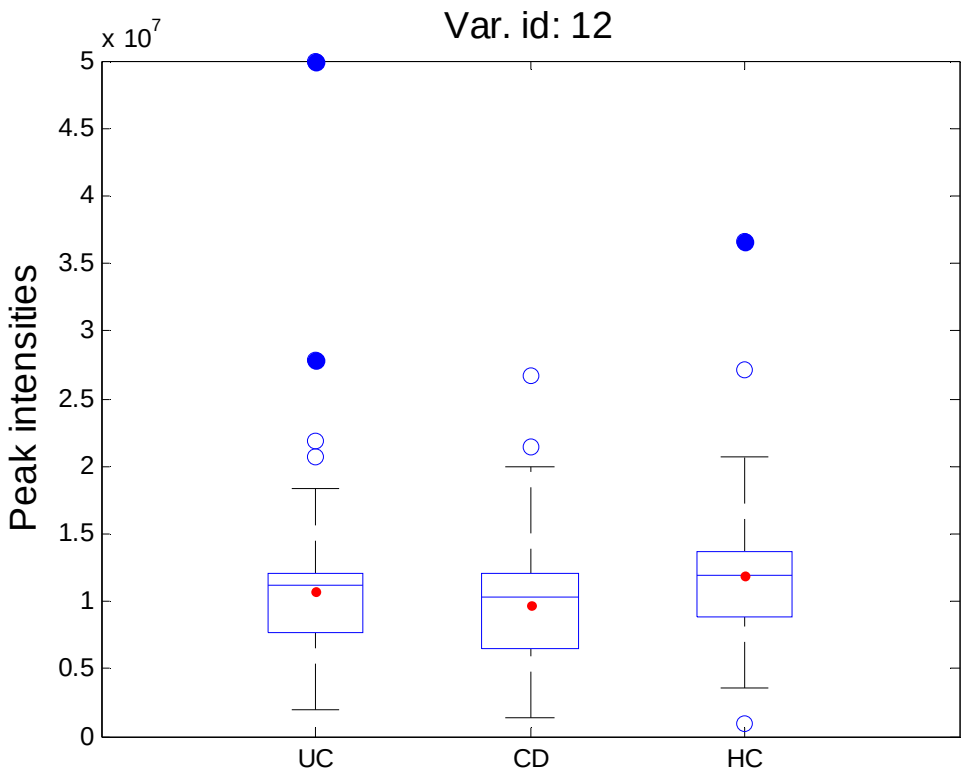
The findings in this experiment, showing raised pyridoxamine or soyasapogenol B 3-O-b-D-glucuronide levels in CD patients, in comparison to UC and healthy controls, may be due to dietary

factors. However, raised soyasapogenol B 3-O-b-D-glucuronide levels in the serum of CD patients may be diagnostic of anti-inflammatory processes occurring in this patient group.

Table 4.29.2: Mass spectra search for 106.0362854 m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB15126	Oxaprozin	M+2H+Na	106.036321	293.105193	0.0000356	Azoles
HMDB33048	Ethyl 1-(propylthio) propyl disulfide	M+2H	106.035807	210.057063	0.0004784	Organic disulphides
HMDB33049	Butyl 1-(methylthio)propyl disulfide	M+2H	106.035807	210.057063	0.0004784	Organic disulphides
HMDB15369	Chlorprothixene	M+3H	106.035559	315.084848	0.0007264	Benzothiopyrans
HMDB12488	1,2,3,4-Tetrahydro-beta-carboline	M+H+K	106.035241	172.100048	0.0010444	Indoles and derivatives
HMDB03929	5-Aminoimidazole	M+Na	106.037565	83.048347	0.0012796	Azoles
HMDB29862	Cyromazine	M+2Na	106.037565	166.096694	0.0012796	Triazines
HMDB40578	4-Thiocyanatophenol	M+2Na+H	106.038024	151.009184	0.0017386	Benzene and substituted derivatives
HMDB34413	1,2-Benzisothiazol-3(2H)-one	M+2Na+H	106.038024	151.009184	0.0017386	Benzothiazoles
HMDB06029	N-Acetylglutamine	M+H+Na	106.0381	188.079707	0.0018146	Carboxylic acids and derivatives

Figure 4.42: Boxplot Urinary Variable ID 12 UC v CD v HC UHPLC-FTMS



p=7.96E-05

In this experiment, urinary peak intensities of variable ID 12 are higher in HCs than in UC or CD. The following metabolites are deemed biologically relevant; N-Acetylglutamine, Ethyl 1-(propylthio)propyl disulfide, Butyl 1-(methylthio)propyl disulfide, 4-Thiocyanatophenol and 5-Aminoimidazole.

N-Acetylglutamine, Ethyl 1-(propylthio)propyl disulfide, Butyl 1-(methylthio)propyl disulfide and 4-Thiocyanatophenol

A recent study investigating the effects of the Mediterranean diet, a diet considered beneficial for health, on the urinary metabolome, has shown increased levels of N-Acetylglutamine when compared to a low fat control diet (Vazquez-Fresno, Llorach et al. 2015). In our study, interestingtly, we see lower levels of N-Acetylglutamine in both disease states when compared to HCs. This is also true for Ethyl 1-(propylthio)propyl disulfide, Butyl 1-(methylthio)propyl disulfide and 4-Thiocyanatophenol, all of which are metabolites from fruits and vegetables. It has been proposed that the typical "Western" diet, low in fruit and vegetables, and high in fat and protein may be associated with IBD. A large systematic review encompassing nineteen studies and 2609 patients revealed that a high intake of fruits is associated with a decreased risk of CD, and a high vegetable intake with a decreased risk of UC (Hou, Abraham et al. 2011). Over half of IBD patients have been shown to place dietary

restrictions upon themselves and up to 45% avoid specifics foods including fruit and vegetables (Limdi, Aggarwal et al. 2015).

5-Aminoimidazole

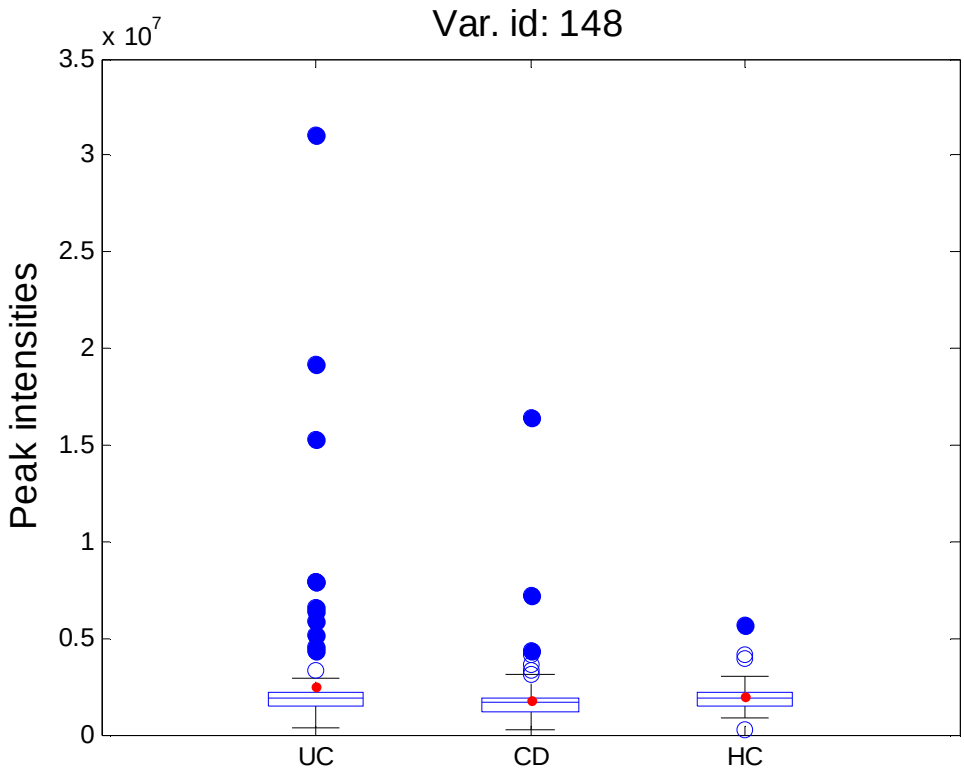
5-Aminoimidazole belongs to the class of organic compounds known as aminoimidazoles. 5-aminoimidazole-4-carboxamide ribonucleoside induces AMP-activated protein kinase activation and has been shown to have a therapeutic effect in ameliorating acute and chronic DSS-induced murine colitis as shown by reduced body weight, loss and significant attenuation in clinical symptoms, and histological inflammation. Also, 5-aminoimidazole-4-carboxamide ribonucleoside treatment inhibits NF-kB activation in macrophages, reduces levels of Th1- and Th17-type cytokines in colon tissues, and down-regulates Th1 and Th17 cell responses during the progress of acute and chronic experimental murine colitis (Bai, Yong et al. 2010). Higher levels in the urine of healthy controls than IBD patients may represent IBD patients not excreting in an attempt to utilise, or higher overall levels in healthy controls.

Table 4.29.3: Mass spectra search for 149.011087 m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB33156	Methoxypyrazine	M+K	149.011171	110.048013	0.000084	Diazines
HMDB03905	Imidazole-4-acetaldehyde	M+K	149.011171	110.048013	0.000084	Azoles
HMDB60741	3-Hydroxy-4-aminopyridine	M+K	149.011171	110.048013	0.000084	Pyridines and derivatives
HMDB38677	Alfafuran	M+H+K	149.011629	258.052823	0.000542	2-arylbenzofuran flavonoids
HMDB33911	5,6,8-Trihydroxy-2-methylbenzo[g]chromen-4-one	M+H+K	149.011629	258.052823	0.000542	Naphthopyrans
HMDB31898	Porric acid C	M+H+K	149.011629	258.052823	0.000542	Benzofurans
HMDB30831	Alternariol	M+H+K	149.011629	258.052823	0.000542	Coumarins and derivatives
HMDB31760	Gentisin	M+H+K	149.011629	258.052823	0.000542	Benzopyrans
HMDB33088	(±)-2-(3,4-Dihydroxyphenyl)-1,3-benzodioxol	M+H+K	149.011629	258.052823	0.000542	Benzodioxoles

	e-5-carboxaldehyde					
HMDB30871	Isogentisin	M+H+K	149.011629	258.052823	0.000542	Benzopyrans

Figure 4.43: Boxplot Urinary variable ID 148 UC v CD v HC UHPLC-FTMS



p=0.00042576

In this experiment, urinary peak intensities of variable ID 148 are higher in UC than in HCs or CD. Imidazole-4-acetaldehyde and alternariol are deemed biologically relevant.

Imidazole-4-acetaldehyde

Imidazole-4-acetaldehyde is a naturally occurring aldehyde metabolite of histamine formed by the action of histaminase, and can be synthesised by oxidation of histidine. There are four histamine receptors known, which all belong to the class of G-protein coupled receptors: histamine H1 receptor (H1R), H2R, H3R and H4R. The H4R is expressed mainly on immune cells such as mast cells, dendritic cells, T-cells and eosinophils, and receptor stimulation leads to a Gi-mediated activation of these cells via phospholipase C activation and subsequent intracellular calcium mobilisation and inhibition of membrane-bound adenylyl cyclase (Schirmer, Rezniczek et al. 2015). Histamine is known to act as a proinflammatory mediator in IBD, with colonic tissues of IBD patients showing increased histamine concentrations as well as higher numbers and activities of mast cells (Raithel,

Matek et al. 1995). H4R blockade is beneficial in TNBS-induced colitis in rats (Varga, Horvath et al. 2005), and in DSS-induced colitis in mice (Schirmer, Rezniczek et al. 2015).

This may explain the raised levels of Imidazole-4-acetaldehyde in UC patients in comparison to CD patients and HCs, in whom colonic disease is not as prevalent.

Alternariol

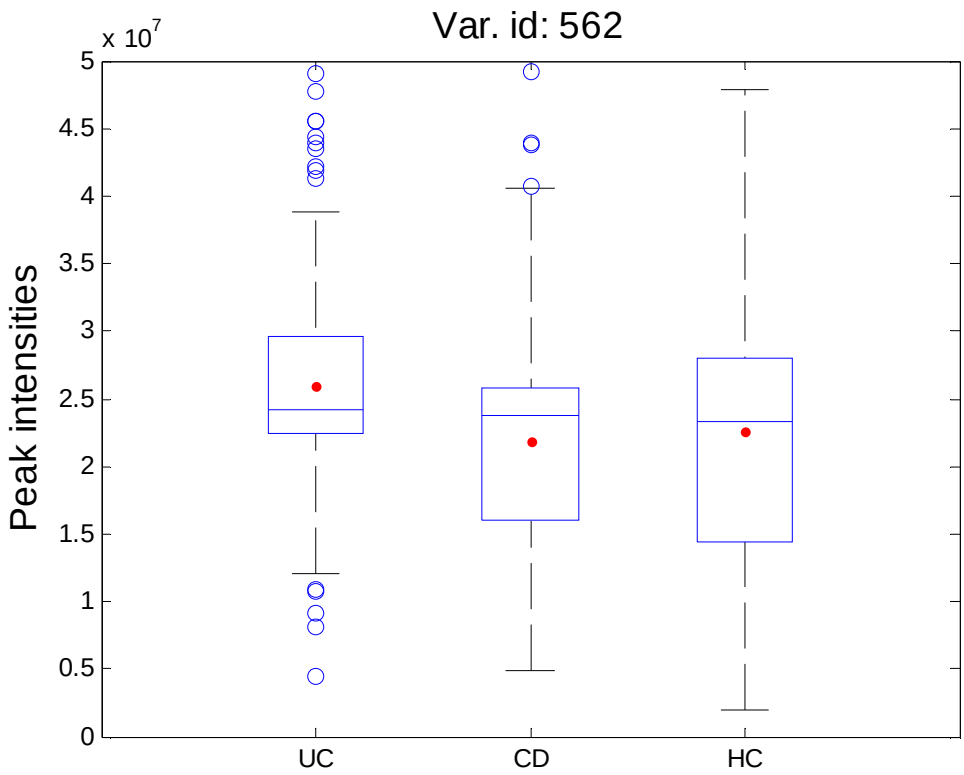
Alternariol, a mycotoxin produced by *Alternaria fungi*, has been shown to change the cell morphology of human macrophages, and to increase the secretion of TNFα and IL-6, as well as reducing macrophage endocytic activity and autophagosomes through the induction of DNA damage (Solhaug, Wisbech et al. 2015). This metabolite may be relevant in the inflammatory and autophagy cycles in IBD patients.

Table 4.29.4: Mass spectra search for 243.1580154 m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB41232	8-Hydroxyhesperetin 7-[6-acetylglucosyl-(1->2)-glucoside]	M+H+2Na	243.157695	682.174514	0.0003204	Flavonoids
HMDB38540	Goshonoside F2	M+2H	243.159085	484.303618	0.0010696	Not classified
HMDB38736	(3S,5R,6R,7E)-3,5,6-Trihydroxy-7-megastigmen-9-one	M+H	243.159085	242.151809	0.0010696	Not classified
HMDB38539	Goshonoside F1	M+2H	243.159085	484.303618	0.0010696	Not classified
HMDB30987	2-Carboxy-4-dodecanolide	M+H	243.159085	242.151809	0.0010696	Not classified
HMDB37163	1,2-Bis(1-ethoxyethoxy)propane	M+Na	243.156677	220.167459	0.0013384	Ethers
HMDB29341	Ceanothine D	M+2H	243.159754	484.304956	0.0017386	Carboxylic acids and derivatives
HMDB15301	Guanethidine	M+2Na-H	243.155607	198.184447	0.0024084	Guanidines
HMDB12557	13'-Carboxy-gamma-tocopherol	M+H+K	243.155022	446.33961	0.0029934	Prenol lipids
HMDB32107	(3beta,5alpha,6beta,7alpha,22E,24	M+H+K	243.155022	446.33961	0.0029934	Steroids and steroid derivatives

	R)-Ergosta-8,22-diene-3,5,6,7-tetrol					

Figure 4.44: Boxplot Urinary Variable ID 562 UC v CD v HC UHPLC-FTMS



p=4.20E-05

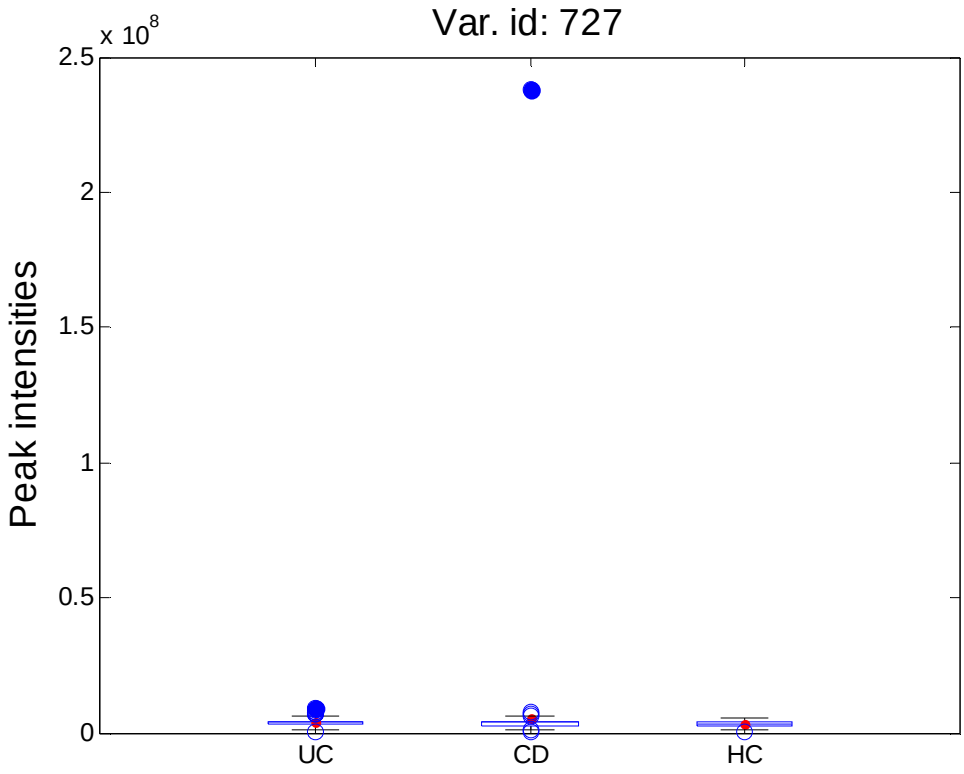
These compounds represent metabolites found in tea, fruits, herbs and spices, milk and milk products, flavouring agents in orange drinks, and mushrooms. This wide range of dietary products is likely to be the cause of the wide ranges of intensities of metabolites seen throughout these groups.

Table 4.29.5: Mass spectra search for 296.1481922m/z

Compound	**Name**	**Adduct**	**Adduct MW (Da)**	**Compound MW (Da)**	**Delta**	**Class**
HMDB59787	Casomorphin	M+2H+Na	296.148892	863.442906	0.0006998	Not classified
HMDB02513	Lithocholate 3-O-glucuronide	M+H+K	296.150134	552.329833	0.0019418	Steroids and steroid derivatives
HMDB01097	Protoporphyrinogen IX	M+H+Na	296.150725	568.304956	0.0025328	Tetrapyrroles and derivatives
HMDB34101	Adouetine Y	M+H+Na	296.150725	568.304956	0.0025328	Not classified

HMDB29342	Ceanothine E	M+H+Na	296.150725	568.304956	0.0025328	Not classified
HMDB30795	Moreollin	M+2H	296.15126	590.287968	0.0030678	Not classified
HMDB00810	Dimethylprotoporphyrin IX dimethyl ester	M+2H	296.151929	590.289306	0.0037368	Tetrapyrroles and derivatives
HMDB05037	Sumatriptan	M+H	296.142724	295.135448	0.0054682	Indoles and derivatives
HMDB34084	Canesceol	M+H+Na	296.142428	568.288362	0.0057642	Steroids and steroid derivatives
HMDB39773	11-alpha-O-beta-D-Glucopyranosyl-16alpha-O-methylneoquassin	M+H+Na	296.142428	568.288362	0.0057642	Prenol lipids

Figure 4.45: Boxplot Urinary Variable ID 727 UC v CD v HC UHPLC-FTMS



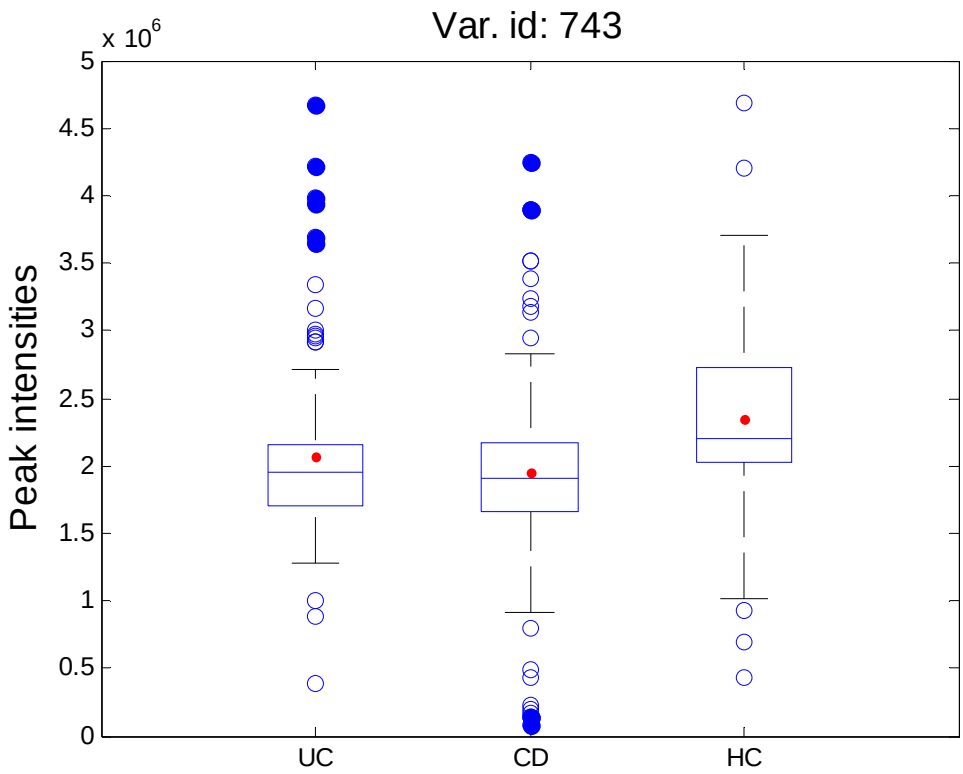
p=0.00033741

These compounds represent metabolites found in milk protein, bile acid, tea, fruits, and in the metabolism of heme synthesis. They are not biologically relevant to IBD.

Table 4.29.6: Mass spectra search for 302.1587031 m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB41889	Etamiphylline	M+Na	302.158743	279.169525	0.0000399	Not available (Super Class Alkaloids and derivatives)
HMDB15385	Lisdexamfetamine	M+K	302.16292	263.199762	0.0042169	Carboxylic acids and derivatives
HMDB30459	Hordatine B	M+H+Na	302.15433	580.312166	0.0043731	2-arylbenzofuran flavonoids
HMDB15237	Sibutramine	M+Na	302.164596	279.175378	0.0058929	Benzene and substituted derivatives
HMDB14339	Tramadol	M+K	302.151687	263.188529	0.0070161	Benzene and substituted derivatives
HMDB15646	Desvenlafaxine	M+K	302.151687	263.188529	0.0070161	Not classified
HMDB60532	O-Desmethylvenlafaxine	M+K	302.151687	263.188529	0.0070161	Not classified
HMDB29567	Hydroxy-alpha-sanshool	M+K	302.151687	263.188529	0.0070161	Alcohols and polyols
HMDB13892	N-Desmethylvenlafaxine	M+K	302.151687	263.188529	0.0070161	Not classified
HMDB15273	Doxepin	M+Na	302.151532	279.162314	0.0071711	Benzoxepines

Figure 4.46: Boxplot Urinary Variable ID 743 UC v CD v HC UHPLC-FTMS



p=2.38E-05

In this experiment, urinary peak intensities of variable ID 743 are increased in HCs compared to UC and CD. Interestingly, the majority of metabolites here are found in drugs, yet in the HC group the peak intensity of this group of compounds is increased in relation to IBD groups. However, Hordatine B, found in barley, may be more prevalent in the HC group in keeping with the theory that in IBD there is reduced dietary variance and increased fat and sugar intake compared to natural fibre intake.

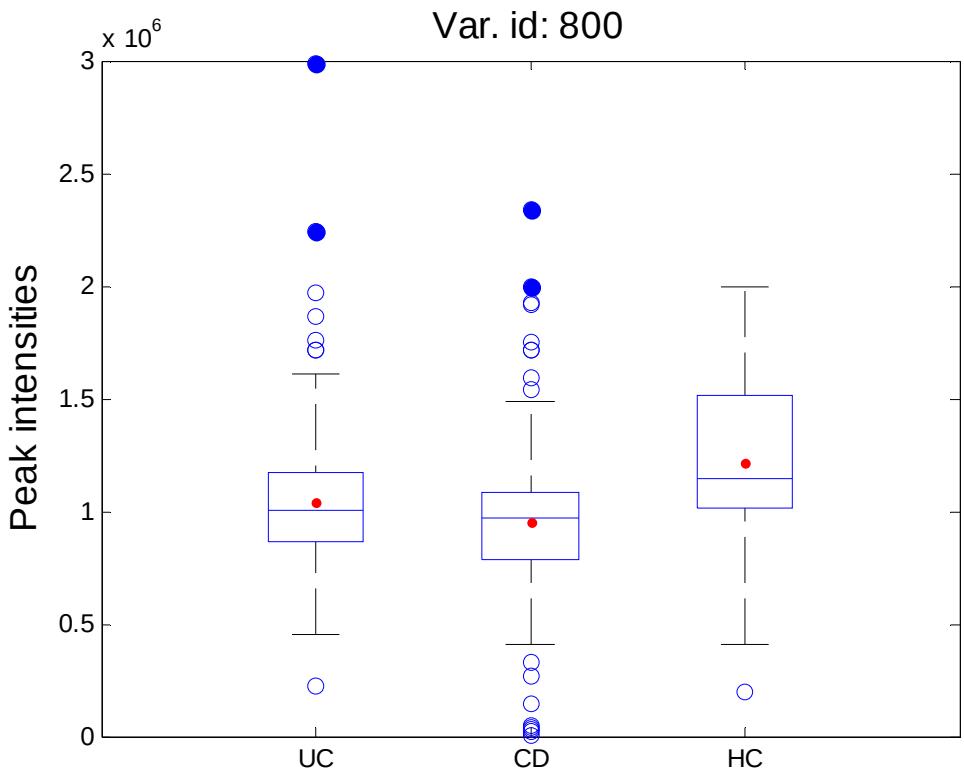
Hydroxy-alpha-sanshool

Hydroxy-alpha-sanshool, naturally found in Japanese pepper, and one of the main active compounds in TU-100, a pharmaceutical grade traditional Japanese medicine, has been shown to accelerate colonic emptying (Manabe, Camilleri et al. 2010), especially in post-operative ileus (Tokita, Yuzurihara et al. 2007) and adhesive intestinal obstruction (Tokita, Yamamoto et al. 2011). At present there are trials underway in the United States of America and Japan on the use of TU-100 in patients with post-operative ileus, functional constipation, irritable bowel disease, and Crohn's Disease (Kubota, Ohtake et al. 2015).

Table 4.29.7: Mass spectra search for 334.0909909 m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB30704	Taxiphyllin	M+Na	334.08972	311.100502	0.0012709	Carbohydrates and carbohydrate conjugates
HMDB60471	Dhurrin	M+Na	334.08972	311.100502	0.0012709	Carbohydrates and carbohydrate conjugates
HMDB37841	N-(1-Deoxy-1-fructosyl)methionine	M+Na	334.093091	311.103873	0.0021001	Carbohydrates and carbohydrate conjugates
HMDB15585	Chlophedianol	M+2Na-H	334.094502	289.123342	0.0035111	Benzene and substituted derivatives
HMDB60463	Citalopram propionic acid	M+Na	334.08499	311.095772	0.0060009	Not classified
HMDB14045	3-Methoxymorphinan	M+2K+H	334.097004	257.177964	0.0060131	Morphinans
HMDB60552	Dextrorphan	M+2K+H	334.097004	257.177964	0.0060131	Morphinans
HMDB14992	Levorphanol	M+2K+H	334.097004	257.177964	0.0060131	Morphinans
HMDB39087	Sudachiin B	M+2H	334.097076	666.1796	0.0060851	Flavonoids
HMDB39088	Sudachiin C	M+2H	334.097076	666.1796	0.0060851	Not classified

Figure 4.47: Boxplot Urinary Variable ID 800 UC v CD v HC UHPLC-FTMS



p=1.03E-05

In this experiment, urinary peak intensities of variable ID 800 are increased in HCs compared to UC and CD. Taxiphyllin, dhurrin and N-(1-Deoxy-1-fructosyl)methionine are carbohydrate derivatives from plants. Sudachiin B and C are found in citrus fruits. These are seen to be more abundant in healthy controls than in IBD patients, as we would expect.

4.8.4 Experiment 6.2: Metabolite Identification UC v CD v HC GC-ToF-MS

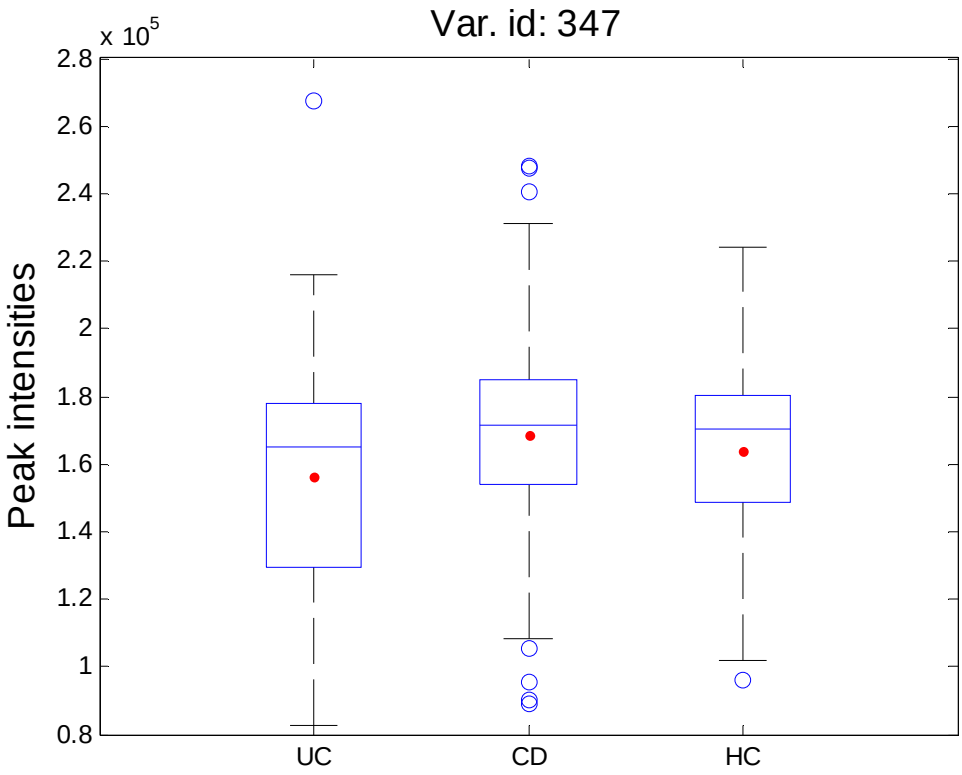
In this experiment using the GC-ToF-MS platform, in-house library spectra with the help of retention indices, as well as the NIST libraries are used to identify putative matches of metabolites. As in Experiment 6.1, each identified metabolite is investigated to determine biological relevance in the clinical setting described (in this experiment considering the differences between UC, CD and HC). Where variable IDs repetitively match to the same metabolite, only one blox plot is shown to represent the diffences seen between groups.

Table 4.30: Important Putative Metabolites UC v CD v HC GC-ToF-MS

Variable ID	Biofluid	Quant mass	Retention time	Putative match	p value	q value
347	Urine	277	786.4035	Propanetric arboxylic acid	0.0021874	0.043748
8	Serum	47	573.478	Unknown	2.38E-05	0.0075684
14	Serum	49	577.428	Unknown	1.14E-05	0.0036252
27	Serum	52	571.628	Threonine or urea	1.55E-05	0.004929
30	Serum	53	575.774	Unknown	1.27E-05	0.0040386
45	Serum	56	574.878	Unknown	3.99E-06	0.00126882
60	Serum	60	570.318	Urea	1.14E-05	0.0036252
64	Serum	61	575.528	Unknown	4.76E-05	0.0151368
69	Serum	63	576.428	Unknown	6.82E-06	0.00216876
74	Serum	64	570.778	Urea	3.18E-06	0.00101124
76	Serum	65	571.903	Threonine or urea	9.70E-07	0.00030846
81	Serum	66	571.278	Threonine or urea	2.69E-06	0.00085542
86	Serum	67	572.201	Unknown	1.99E-06	0.00063282
93	Serum	69	571.028	Threonine or urea	2.25E-06	0.0007155
98	Serum	70	561.694	Dihydroxybutanoic acid or Serine	2.32E-06	0.00073776
106	Serum	71	571.978	Threonine or urea	3.35E-06	0.0010653
107	Serum	72	572.378	Unknown	4.00E-06	0.001272
121	Serum	75	574.929	Unknown	5.13E-05	0.0163134
133	Serum	78	572.928	Unknown	3.90E-06	0.0012402
137	Serum	79	570.426	Urea	5.90E-05	0.018762
142	Serum	80	570.674	Urea	8.32E-06	0.00264576
157	Serum	84	575.428	Unknown	6.72E-05	0.0213696
218	Serum	99	570.728	Urea	1.30E-06	0.0004134
221	Serum	100	562.088	Dihydroxybutanoic acid or Serine	2.18E-05	0.0069324
228	Serum	102	563.626	Dihydroxybutanoic acid or Serine	0.00012686	0.04034148
266	Serum	111	571.579	Threonine or urea	3.48E-06	0.00110664

279	Serum	115	572.628	Unknown	1.70E-05	0.005406
334	Serum	127	571.479	Threonine or urea	1.45E-05	0.004611
355	Serum	132	576.428	Unknown	1.03E-05	0.0032754
390	Serum	141	570.928	Urea	4.04E-06	0.00128472
424	Serum	150	579.278	Unknown	4.11E-05	0.0130698
428	Serum	151	580.351	Unknown	0.00014196	0.04514328
446	Serum	155	570.524	Urea	1.94E-06	0.00061692
455	Serum	157	570.478	Urea	3.97E-06	0.00126246
507	Serum	171	570.474	Urea	2.96E-06	0.00094128
509	Serum	172	570.678	Urea	3.81E-06	0.00121158
513	Serum	173	570.328	Urea	2.47E-06	0.00078546
563	Serum	186	570.728	Urea	1.24E-05	0.0039432
569	Serum	187	571.628	Threonine or urea	2.63E-05	0.0083634
574	Serum	189	570.078	Urea	2.33E-06	0.00074094
576	Serum	190	569.928	Urea	2.60E-06	0.0008268
582	Serum	191	569.826	Urea	1.94E-05	0.0061692
614	Serum	198	954.428	Unknown	0.00012029	0.03825222
844	Serum	265	845.578	Fructose	3.60E-05	0.011448
921	Serum	292	657.578	Aminomalonic acid	3.94E-05	0.0125292
960	Serum	305	825.874	Eicosane	1.38E-06	0.00043884
990	Serum	318	829.026	Glucuronic acid	1.51E-06	0.00048018
1056	Serum	347	795.178	Pentanoic acid	0.00010806	0.03436308
1131	Serum	375	790.151	Galactose or Cellobiose	9.97E-05	0.0317046
1325	Serum	432	841.678	Myo-inositol	0.00015209	0.04836462

Figure 4.48: Boxplot Urinary Variable ID 347 Propanetricarboxylic acid UC v CD v HC GC-ToF-MS



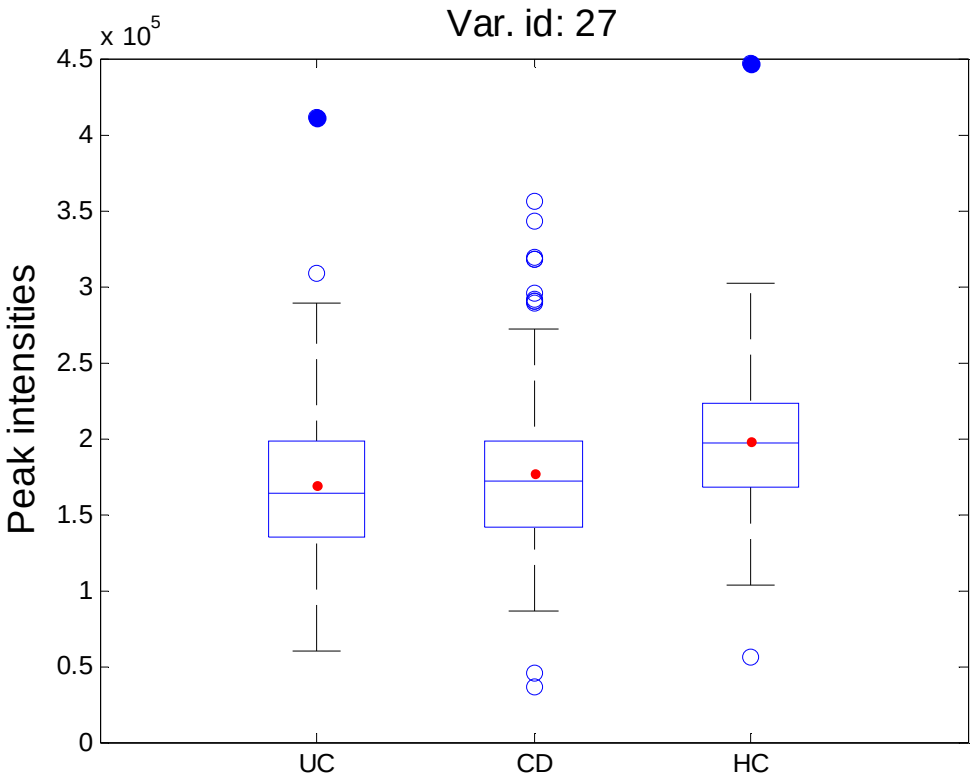
p=0.0021874

In this experiment, urinary peak intensities of variable ID 347 (Propanetricarboxylic acid) are increased in CD compared to HCs and UC.

Propanetricarboxylic acid

Propanetricarboxylic acid, belonging to the class Carboxylic Acids and Derivatives, is an inhibitor of the enzyme aconitase, which catalyses the conversion of cirtic acid into isocitric acid in the citric acid or tricarboxylic acid (TCA) cycle (Kreb's cycle). It has been shown that in UC patients reduced levels of amino acids are seen in both sera and colonic tissue, resulting in reduced TCA cycle-related downstream molecules (Ooi, Nishiumi et al. 2011). This is also demonstrated in our UC cohort.

Figure 4.49: Boxplot Serum Variable ID 27 Threonine or Urea UC v CD v HC GC-ToF-MS



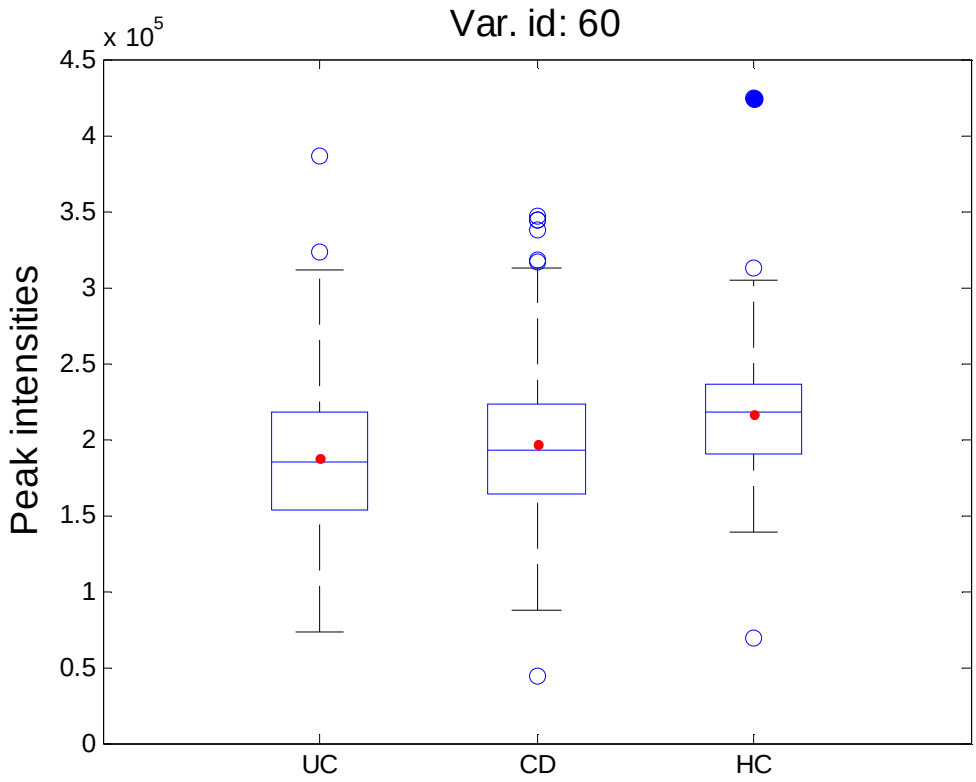
p=1.55E-05

In this experiment, all of the serum variables identified as threonine or urea show increased peak intensities in HCs compared to IBD patients.

Threonine

Threonine is an essential amino acid, belonging to the class Fatty Acyls, is abundant in human plasma, especially in the newborn. It is an immunostimulant, promoting thymus gland growth and promoting cell immune defence function. Threonine is required for the production and maintenance of mucin in the gut, and the rate of mucin synthesis has been shown to be dircectly related to the availability of dietary threonine in both rats (Faure, Moennoz et al. 2005) and piglets (Law, Bertolo et al. 2007). In a rat model of sepsis, threonine utilisation for the synthesis of intesitinal mucins was 70% greater than in healthy controls (Faure, Chone et al. 2007). In ileitis and colitis models, intestinal mucin production is not stimulated in pair-fed animals and only dietary supply of threonine, serine, proline and cysteine was effective in promoting colonic mucin synthesis, with threonine being shown to be the rate limiting amino-acid for mucin synthesis in the intestinal mucosa (Faure, Mettraux et al. 2006, Remond, Buffiere et al. 2009). Our IBD groups show reduced threonine levels compared to HCs. Currently there is a study recruiting with the aim of identifying threonine requirement in those with CD, UC and HCs (https://clinicaltrials.gov/ct2/show/NCT02423460).

Figure 4.50: Boxplot Serum Variable ID 60 Urea UC v CD v HC GC-ToF-MS

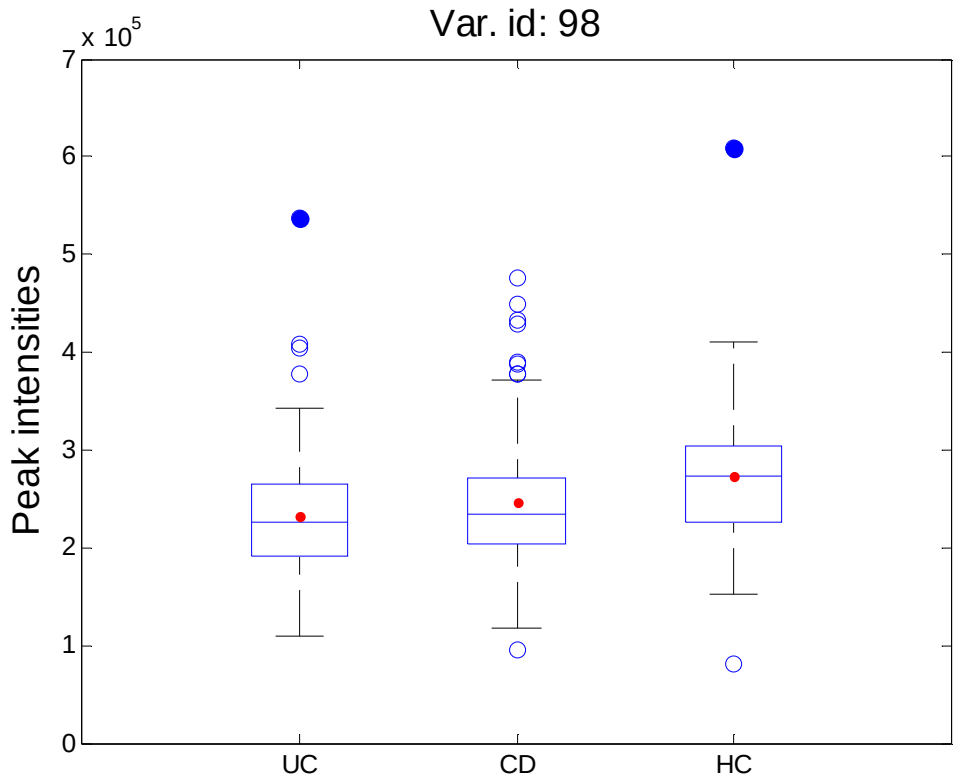


p=1.14E-05

Urea

Urea is a highly soluble organic compound formed in the liver from ammonia produced by the deamination of amino acids. It is excreted in urine by the kidney. During the study all participants were fasted for a minimum of six hours. However, they were allowed to drink water during this period. The individual states of hydration were not measured or standardised and therefore we do not class reduced urea levels in IBD patients compared to HCs as relevant to disease pathogenesis.

Figure 4.51: Boxplot Serum Variable ID 98 Dihydroxybutanoic acid or Serine UC v CD v HC GC-ToF-MS



p=2.32E-06

In this experiment all of the serum variables identified as Dihydroxybutanoic acid or Serine have peak intensities greater in HCs than in IBD patients.

Dihydroxybutanoic acid

Dihydroxybutanoic acid, belonging to the class Fatty Acyls, an organic acid that is a major component of cerebrospinal fluid (CSF), has been investigated as a potential biomarker in Alzheimer's Disease (Oresic, Hyotylainen et al. 2011, Mousavi, Jonsson et al. 2014). This metabolite has been shown to be overproduced under low oxygen conditions from D-galacturonic acid, a uronic acid, which is a stereoisomer of glucoronic acid. D-galacturonic acid, a sugar acid, is the main component of pectin. Dietary pectin has been shown to down regulate colonic inflammatory responses by moderating the production of proinflammatory cytokines and immunoglobulins (Ye, Lim 2010).

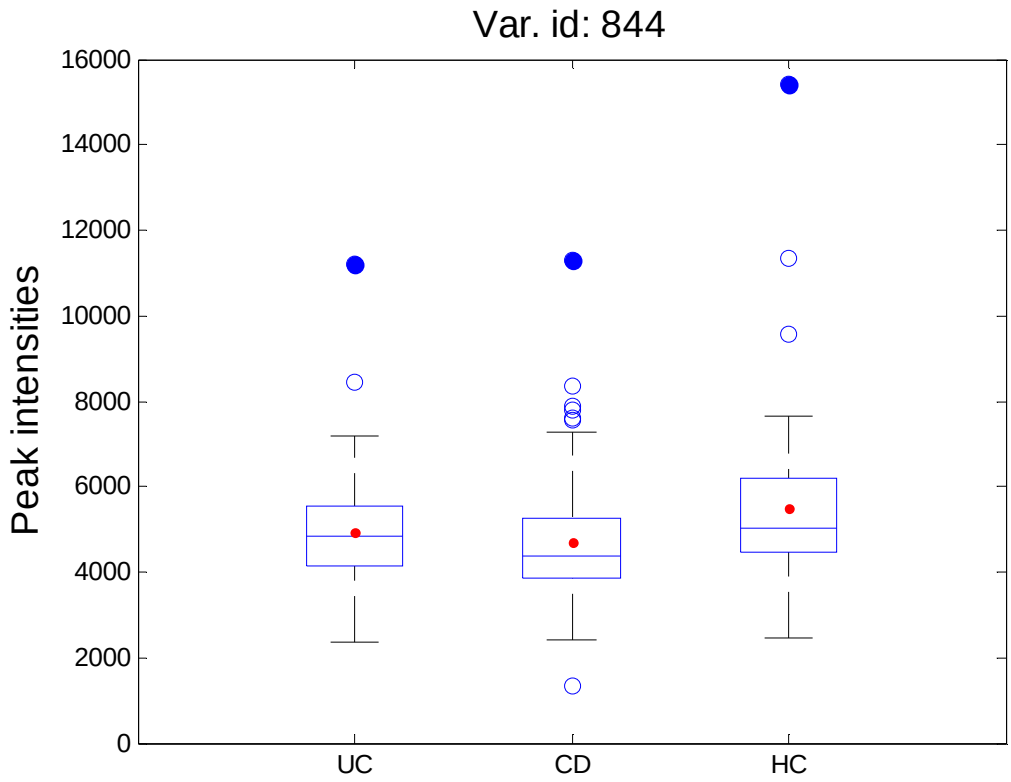
Serine

Serine, belonging to the class Carboxylic Acids and Derivatives, has also been investigated as a biomarker in Alzheimer's Disease (Madeira, Lourenco et al. 2015). Recently theories regarding the multi-factorial-multisystem nature of dementia with inflammation playing a central role have been

considered. Irritable bowel syndrome (IBS) and the associated alteration of the gut microbiome enhance gut inflammation, intestinal barrier dysfunction, and systemic to neuroinflammation may provide key insights. Hence, gut dysbiosis is now beginning to be considered as a potential therapeutic target for prevention of IBS related conditions including congnitive decline (Daulatzai 2014).

Whilst links with IBD and dementia have not yet been established, there are reports of rapidly progressive dementia as a presenting feature in IBD (Papathanasiou, Nikakis et al. 2014).

Figure 4.52: Boxplot Serum Variable ID 844 Fructose UC v CD v HC GC-ToF-MS



p=3.60E-05

In this experiment the serum variables identified as fructose have higher peak intensities in HCs than in IBD patients.

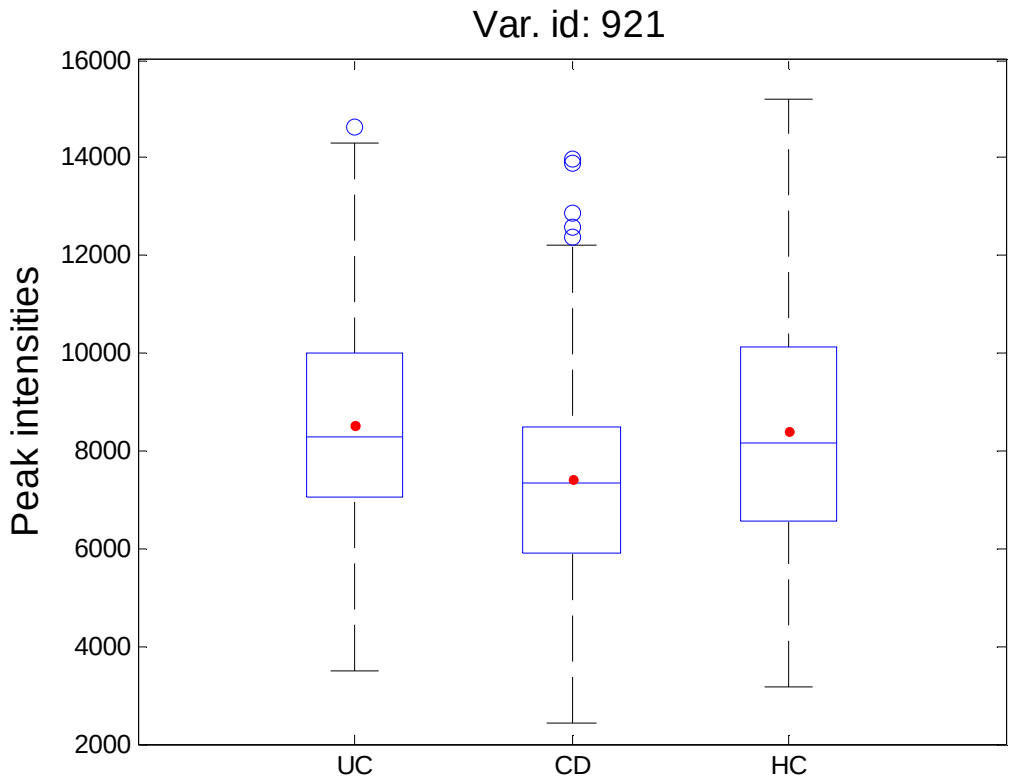
Fructose

Fructose, a simple monosaccharide in the class Carbohydrates and Carbohydrate Conjugates, is found in fruits and berries, root vegetables and honey. A high level of fructose is negatively associated with IBD (Reif, Klein et al. 1997, Mahmud, Weir 2001). In our study HC have higher serum fructose levels than IBD patients, as would be expected.

Prebiotic oliogsaccharides are not sensitive to gastric acid, and remain intact when they reach the colon (Delzenne 2003). They promote the proliferation of beneficial bacteria such as *Bifidobacteria*

and *Lactobacilli* (Bouhnik, Raskine et al. 2004) and have a positive effect in maintaining intestinal homeostasis (Cherbut 2002, Nyman 2002). Fermentation of these carbohydrates produces SCFAs, mainly acetate, propionate, and butyrate (Sanderson 2007). Fructose-based oligo- and polysaccharides have been reported as promising candidates in IBD management (Lara-Villoslada, de Haro et al. 2006).

Figure 4.53: Boxplot Serum Variable ID 921 Aminomalonic acid UC v CD v HC GC-ToF-MS



p=3.94E-05

In this experiment, similar serum levels of the variable identified as aminomalonic acid are seen in HCs and UC, with lower peak intensities in CD.

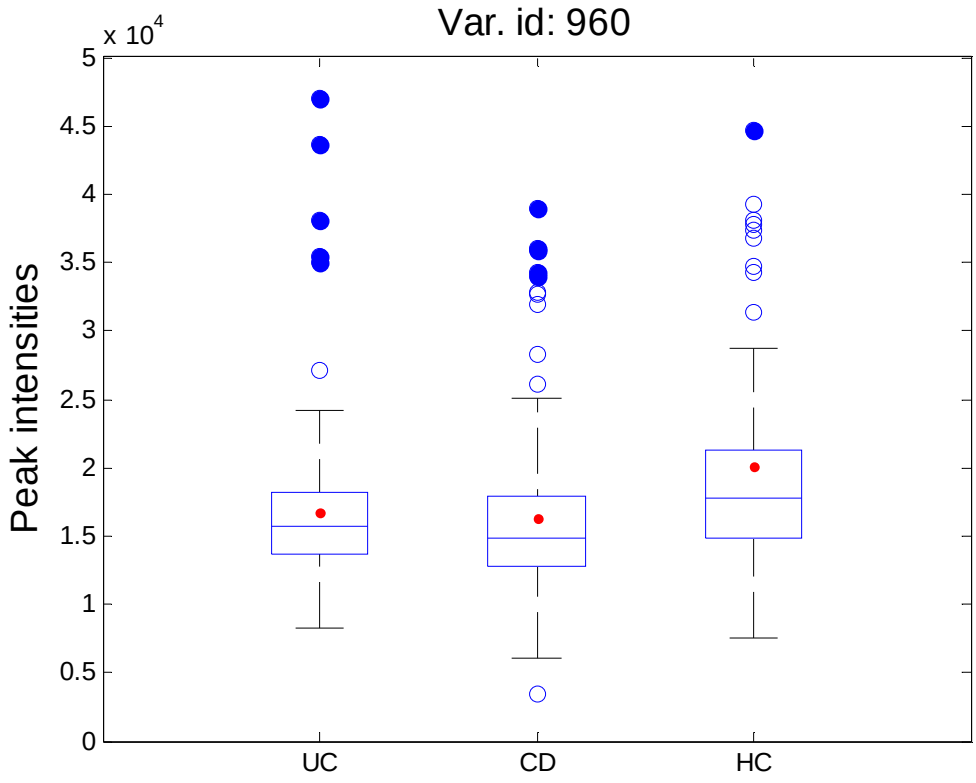
Aminomalonic acid

Aminomalonic acid, a dicarboxylic acid in the class Carboxylic Acids and Derivatives, has been isolated from proteins of *Escherichia coli* and human atherosclerotic plaque (Van Buskirk, Kirsch et al. 1984). Its potential origins are errors of protein synthesis and oxidative damage to amino acid residues in proteins. Aminomalonic acid metabolism is not fully understood.

Aminomalonic acid has been proposed as a potential biomarker in hepatocellular carcinoma as the conversion of cysteine into acidic aminomalonate could induce conformational changes that are important for ubiquitinylation and degradation of iron regulatory protein2 (IRP2). IRP2 regulates

post-transcriptionsal expression of mRNA-encoding proteins involved in iron homeostasis and utilisation, and potentially in tumour cell metabolism and proliferation in HCC (Xue, Lin et al. 2008).
In renal transplant patients with acute allograft rejection, aminomalonic acids levels have been found to be low. When taken in conjuncation with other metabolites this can be ulitised in the diagnosis of acute rejection and also stable function with reasonable accuracy (Mao, Bai et al. 2008).
In the serum of patients with large abdominal aortic aneurysms, aminomalonic acid has been shown to be 800% higher than in control patients (Ruperez, Ramos-Mozo et al. 2012). This may be related to atherosclerosis, however, interestingly a study in patients with acute coronary syndrome has shown low levels of aminomalonic acid (Teul, Garcia et al. 2011).
Recently, reduced levels of aminomalonic acid have been reported in canine IBD (Minamoto, Otoni et al. 2015). In our study, aminomalonic acid is reduced in the serum of CD patients, but increased in the serum of UC patients when compared to HCs. Clearly this metabolite requires further investigation with regards to not only its mechanism of action and metabolic pathways, but also its utilisation as a potential biomarker in many fields.

Figure 4.54: Boxplot Serum Variable ID 960 Eicosane UC v CD v HC GC-ToF-MS



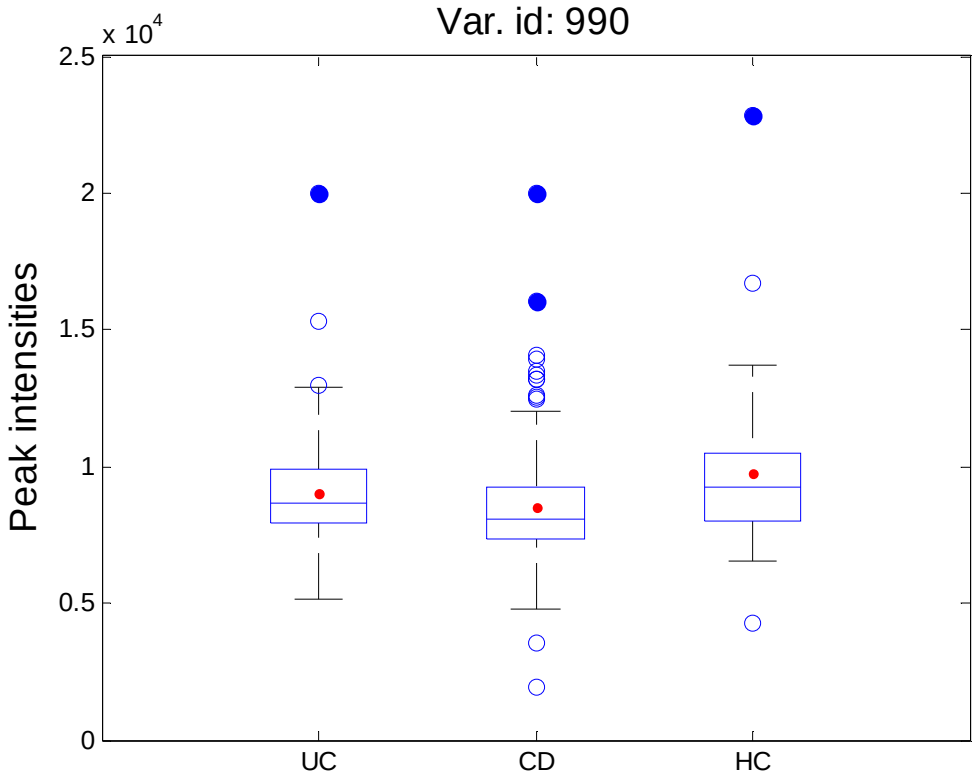
p=1.38E-06

In this experiment higher peak intensities of the serum variable identified as eicosane are seen in HCs compared to IBD patients.

Eicosane

Eicosane is an acyclic hydrocarbon in the Alkane class. There is no known relationship to IBD.

Figure 4.55: Boxplot Serum Variable ID 990 Glucuronic acid UC v CD v HC GC-ToF-MS



p=1.15E-06

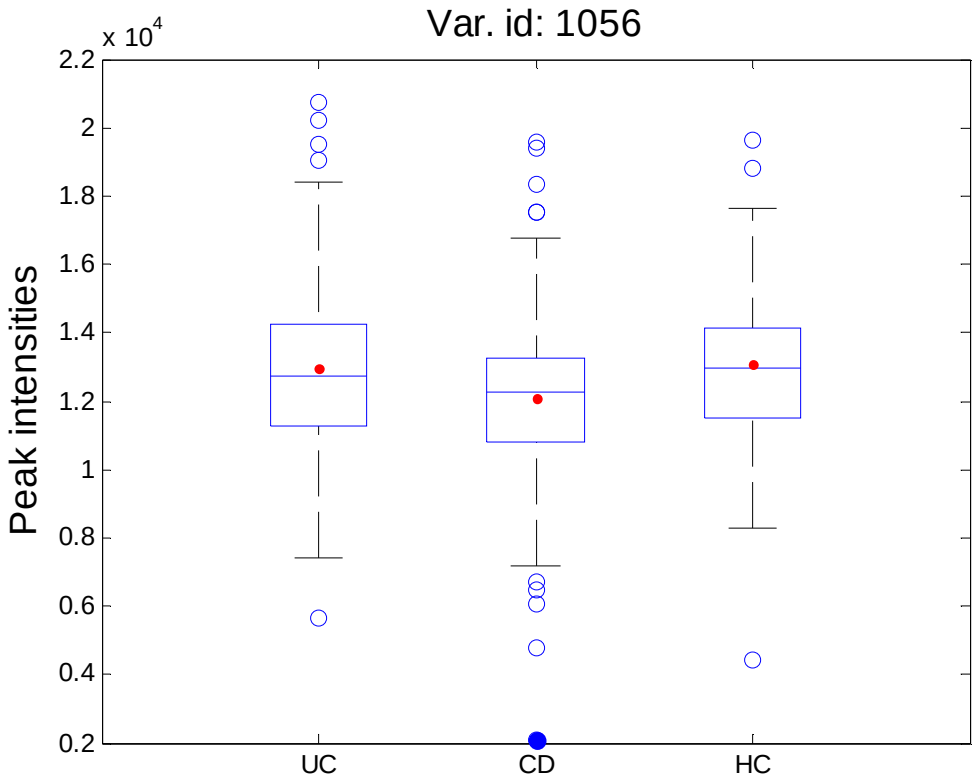
In this experiment the serum variable identified as glucuronic acid has higher peak intensities in HCs than in UC or CD.

Glucuronic acid

Glucuronic acid, or glucuronate, belongs to the Carbohydrate and Carbohydrate Conjugate class. It is synthesised in the uronic acid pathway, an alternative pathway for the oxidation of glucose that does not provide ATP. Instead, the activated form of glucuronate, uridine diphosphate-glucuronate is formed, and is mainly used for detoxification of foreign chemicals and in the synthesis of mucopolysaccharides. Glucuronate is hydrolysed to form glucaronic acid. In certain species this can be synthesised into ascorbic acid (vitamin C). However, in humans, the required enzyme L-gluconolate oxidase is absent and vitamin C must come from the diet. The excess glucaronic acid is utilised by the pentose phosphate pathway. Interestingly, the pentose phosphate pathway is known to be overrepresented in IBD, especially in ileal CD (Morgan, Tickle et al. 2012), which may be the

reason for the low levels of glucaronic acid seen in serum of both UC and especially CD in relation to HCs in our study.

Figure 4.56: Boxplot Serum Variable ID 1056 Pentanoic acid UC v CD v HC GC-ToF-MS



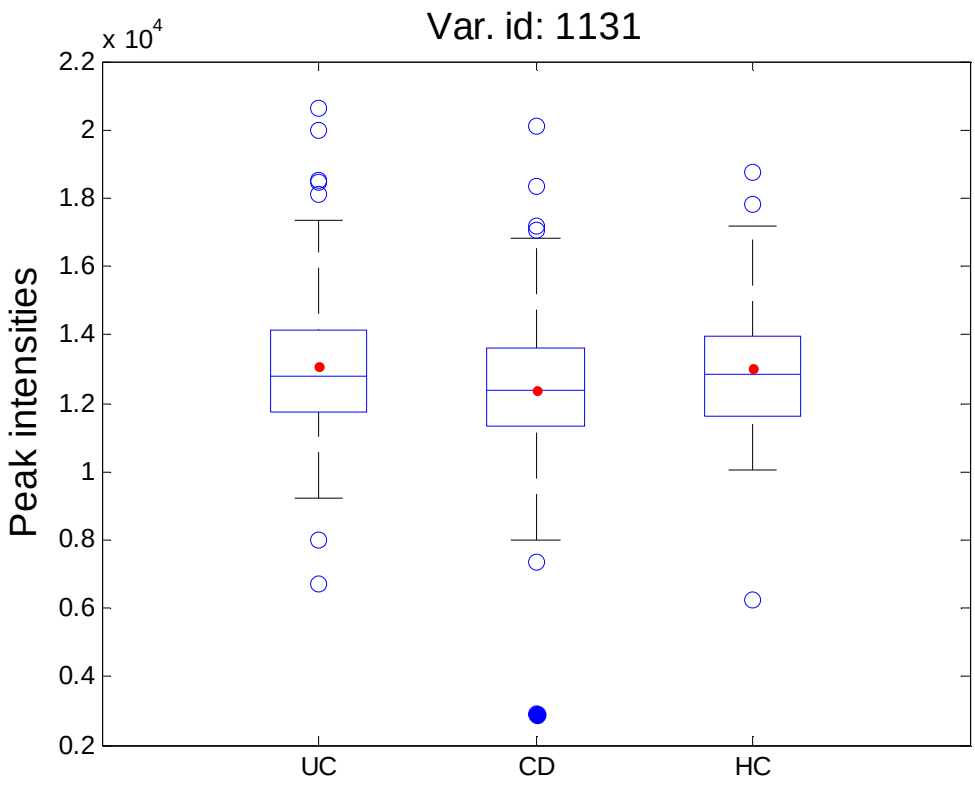
p=0.00010806

In this experiment lower peak intensities of the serum variable identified as pentanoic acid are seen in CD than in UC and HCs.

Pentanoic acid

Pentanoic acid, also known as valeric acid, is a naturally occurring micronutrient in the Fatty Acyls class, which is synthesised in small amounts by plants and animals, including humans. It has antioxidant and chemoprotective properties, acting as a free radical scavenger, assisting in repairing oxidative damage and regenerating endogenous antioxidants including vitamin C, E and glutathione. Glutathione,a key biomarker of oxidative stress, reduces intracellular reactive oxygen species and plays an important role in the detoxification of xenobiotics. This has previously been shown to be reduced in IBD (Iantomasi, Marraccini et al. 1994, Karp, Koch 2006). Recently pentanoate levels have been shown to be reduced in faecal metabolite profiling in IBD (De Preter, Machiels et al. 2015).

Figure 4.57: Boxplot Serum Variable ID 1131 Galactose or Cellobiose UC v CD v HC GC-ToF-MS



p=9.97E-05

In this experiment lower peak intensities of the serum variable identified as either galactose or cellobiose are seen in CD than in UC and HCs.

Galactose

Galactose, belonging to the Carbohydrate and Carbohydrate Conjugates class, is an aldohexose that occurs naturally in the D-form in lactose, cerebrosides, gangliosides, and mucoproteins. D-Galactose is an energy-providing nutrient and also a necessary basic substrate for the biosynthesis of many macromolecules in the body.

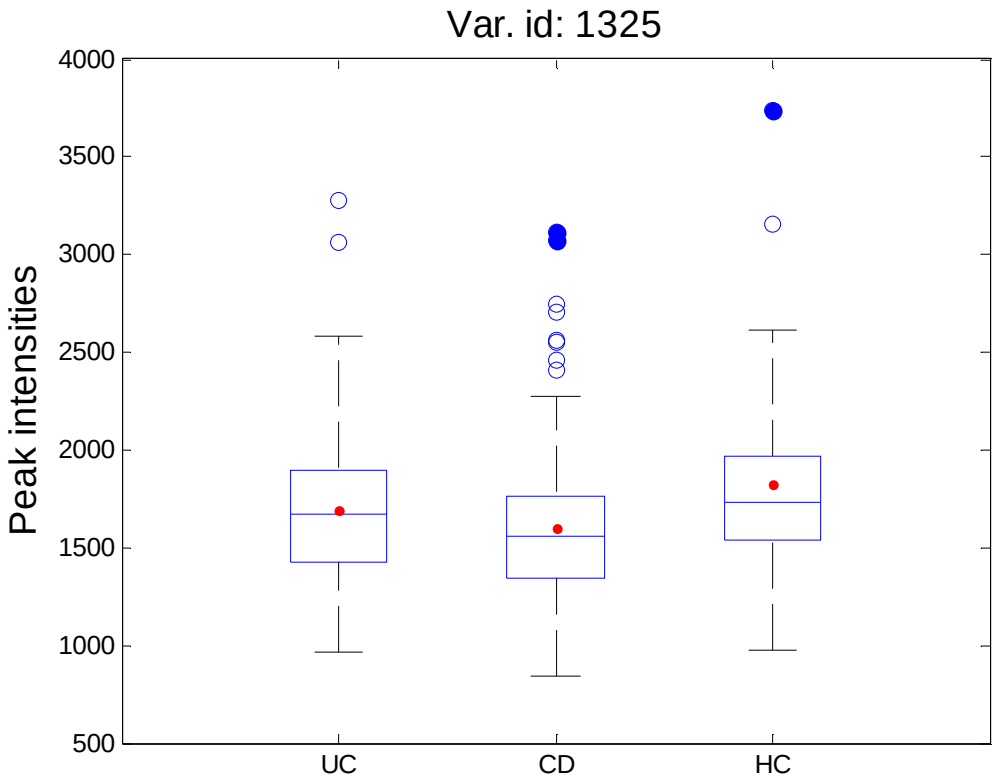
IgG, the most abundant class of antibody in human plasma, provides the majority of antibody-based immunity against pathogens. IgG can demonstrate both pro and anti-inflammatory activity depending on the glycosylation status (Theodoratou, Campbell et al. 2014). The anti-inflammatory activity of IgG is mediated by Fc galactosylation, and decreased IgG galatosylation has been observed in the sera of IBD patients (Dube, Rook et al. 1990, Shinzaki, Iijima et al. 2008). The extent of IgG galactosylation, measured by HPLC, has been considered as a potential biomarker in IBD (Shinzaki, Iijima et al. 2008).

Cellobiose

Cellobiose, belonging to the Carbohydrate and Carbohydrate Conjugates class, is produced from cellulose using bacterial enzymes, and is hydrolysed into two glucose molecules by cellobiosidase and cellulase. In mice with DSS induced colitis, cellobiose appears to have a protective effect (Nishimura, Andoh et al. 2010). It has been suggested that cellobiose fermented by the intestinal microflora acts as a prebiotic (van Zanten, Sparding et al. 2015), but also that it inhibits proinflammatory cytokine expression via the anti-inflammatory actions of SCFAs, especially butyrate (Nishimura, Andoh et al. 2010).

Low levels of these metabolites seen in our study in CD are in keeping with previous studies. These metabolites may aid in the development of biomarkers and therapies in the future.

Figure 4.58: Boxplot Serum Variable ID 1325 Myo-inositol UC v CD v HC GC-ToF-MS



p=0.00015209

In this experiment the serum variable identified as myo-inositol has higher peak intensities in HCs than in UC or CD.

Myo-inositol

Myo-inositol, belonging to the class Alcohols and Polyols, is a product of glycerophospholipid metabolism and is a substrate of D-glucaronate. Lower levels of myo-insitol seen in IBD patients may contribute to the low levels of glucaronate seen in IBD, as discussed previously.

4.8.5 Experiment 6 Summary

Metabolites in the classes Fatty Acyls, Carboxylic Acids and Derivatives, Carbohydrate and Carbohydrate Conjugates, appear reduced in IBD compared to HCs.

4.9 Experiment 7: Metabolite Identification Pre Biological v Post Biological (Grouped)

In this experiment, the IBD patients pre biological treatment are compared to those post biological treatment. The samples are grouped as pre and post biological therapy. Future experiments will study paired samples.

Table 4.31 Experiment 7and 8 number of samples analysed

	Pre Biological	**Post Biological**
Serum samples	24	24
Urine samples	24	23

4.9.1 Experiment 7.1: Metabolite Identification Pre Biological v Post Biological (Grouped) UHPLC-FTMS

Table 4.32: Important Variables Identified Pre Biological Therapy v Post Biological Therapy (Grouped) UHPLC-FTMS

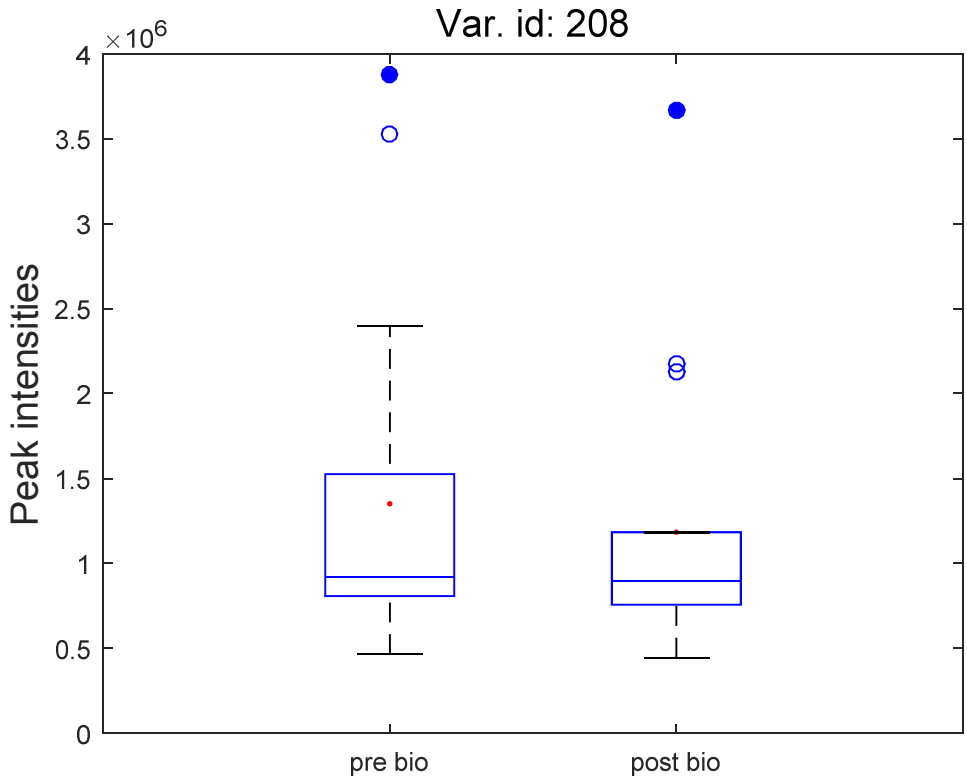
Variable ID	**Biofluid**	**M/z**	**Retention Time**	**p value**	**q value**
208	**Urine**	160.0959935	222.1519	0.0034846	0.0313614

Table 4.32.1: Mass spectra search for 160.0959935 m/z

Compound	**Name**	**Adduct**	**Adduct MW (Da)**	**Compound MW (Da)**	**Delta**	**Class**
HMDB31345	Calystegine A6	M+H	160.096819	159.089543	0.0008255	Tropane alkaloids
HMDB38625	Medicanine	M+H	160.096819	159.089543	0.0008255	Carboxylic acids and derivatives
HMDB29230	4-Hydroxystachydrine	M+H	160.096819	159.089543	0.0008255	Carboxylic acids and derivatives
HMDB12154	3-Dehydrocarnitine	M+H	160.096819	159.089543	0.0008255	Keto acids and derivatives
HMDB11757	N-Acetylvaline	M+H	160.096819	159.089543	0.0008255	Carboxylic acids and derivatives
HMDB38593	Calystegine A3	M+H	160.096819	159.089543	0.0008255	Tropane alkaloids
HMDB00339	2-Methylbutyrylglycine	M+H	160.096819	159.089543	0.0008255	Carboxylic acids and derivatives
HMDB00927	Valerylglycine	M+H	160.096819	159.089543	0.0008255	Carboxylic acids and derivatives
HMDB29409	Turicine	M+H	160.096819	159.089543	0.0008255	Carboxylic acids and derivatives

HMDB36384	Calystegine A7	M+H	160.096819	159.089543	0.0008255	Not classified

Figure 4.59: Boxplot Urinary Variable ID 208 Pre Biological Therapy v Post Biological Therapy (Grouped) UHPLC-FTMS



p=0.0034846

In this experiment the urine variable ID 208 has higher peak intensities in pre biological therapy patients than in post biological therapy patients. Calystegine A3, 3-Dehydrocarnitine and N-acetylvaline are considered biologically relevant.

Calystegine A3

Calystegines, organic tropane alkaloids, which occur in plant families, are classified into three groups; A, B, and C, on the basis of the number of hydroxyl group substitutes on the nortropane ring. Calystegine A3, B1, B2 and C1 inhibit rat liver lysosomal β-glucosidase (Asano, Kato et al. 1997). β-glucosidase is involved in cellulose hydrolysis, eventually converting cellobiose to glucose. As discussed previously, cellobiose is potentially protective against DSS-induced colitis and has anti-inflammatory properties. In our study we see potentially lower levels of calystengine A3 post biological treatment. This may represent less inhibition of β-glucosidase, and thus more conversion of cellobiose to glucose in the post biological group. Also, anti-TNFα therapy is known to decrease glycaemic levels (Parmentier-Decrucq, Duhamel et al. 2009) rather than raise them, and thus it is

unlikely that the differences seen in pre- and post-biological therapy groups purely represent the effects of calystegine A3.

3-Dehydrocarnitine

3-Dehydrocarnitine, a member of the carnitine family, is an intermediate in carnitine degradation. It can be formed from either D-carnitine (biologically inactive) or L-carnitine (biologically active). Carnitine is a quaternary ammonium compound biosynthesised from lysine and methionine. In living cells, carnitine is required for the transport of fatty acids from the cytosol into the mitochondria during the breakdown of lipids. L-carnitine has anti-oxidant activity through the reduction of myeloperoxidase (MPO) and malondialdehyde (MDA), the inhibition of the accumulation of lipid peroxides, the up-regulation of superoxide dismutase and the prevention of glutathione reduction. This suppresses the formation and activation of reactive oxygen species, resulting in NF-κB pathway inhibition (Moeinian, Farnaz Ghasemi-Niri et al. 2013).

However, L-carnitine, which is adundant in red meat, is processed by the enteric microbiota into trimethylamine, the human metabolite of which is trimethylamine-*N*-oxide. Trimethylamine-*N*-oxide is a pro-atherosclerotic metabolite and plasma levels of L-carnitine have infact been associated with cardiovascular disease (Koeth, Wang et al. 2013). Broad-spectrum antibiotics have been shown to reduce the metabolic conversions of L-carnitine to trimethylamine-*N*-oxide (Koeth, Wang et al. 2013), indicating that the gut microbiome is significant in non-GI disease processes such as carnitine-associated atherosclerosis.

In IL-10 gene deficient mice, urinary trimethylamine has been shown to parallel the progression of IBD (Murdoch, Fu et al. 2008) and thus may have potential as a biomarker in the future.

In our study we saw lower levels of urinary 3-Dehydrocarnitine after biological treatment. This may represent less carnitine degradation and excretion, as less is present due to the NF-κB pathway being successfully inhibited by TNF-α blockade instead of the body utilising carnitine in this way.

N-acetylvaline

N-acetylvaline is a derivative of valine. Whilst no direct links are seen with this amino acid carrying a N-acylated aliphatic chain, γ-glutamyl carrying valine and cysteine have been investigated with regards to GI homeostasis. The extra-cellular calcium sensing receptor (CaSR) is distributed throughout the GI tract, and its activation has been shown to promote intestinal homeostasis. The γ-glutamyl dipeptides, γ-glutamyl cysteine, and γ-glutamyl valine, are dietary flavour enhancing compounds that can activate CaSR via allosteric ligand binding to exert anti-inflammatory effects in the GI tract by blocking the activation of the TNF-α-dependent pro-inflammatory signalling cascade (Zhang, Kovacs-Nolan et al. 2015). In our study we saw lower levels of urinary N-acetylvaline after biological treatment. This may represent less excretion of valine derivatives, which promote anti-inflammatory effects in the gastrointestinal tract.

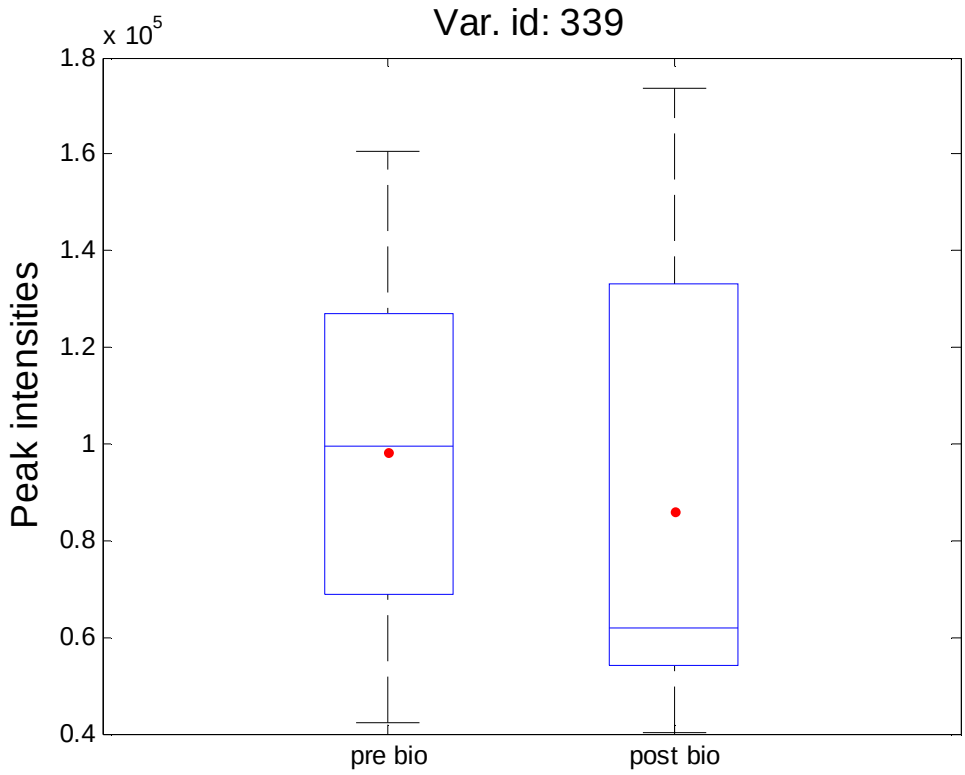
4.9.2 Experiment 7.2: Metabolite Identification Pre Biological v Post Biological (Grouped) GC-ToF-MS

As in Experiment 7.1, the samples are grouped as pre and post biological therapy. Future experiments will study paired samples.

Table 4.33: Important Putative Metabolites Pre Biological Therapy v Post Biological Therapy (Grouped) GC-ToF-MS

Variable ID	Biofluid	Quant mass	Retention time	Putative match	p value	q value
339	Urine	265	882.328	Acetamide	0.023985	0.023985

Figure 4.60: Boxplot Urinary Variable ID 339 Acetamide Pre Biological Therapy v Post Biological Therapy (Grouped) GC-ToF-MS



p=0.023985

In this experiment lower peak intensities of the urinary variable identified as acetamide were identified in post biological therapy patients than in pre biological therapy patients.

Acetamide

Acetamide, the amide of acetic acid (commonly used to induce murine colitis (Randhawa, Singh et al. 2014)), is found in red beetroot. Belonging to the family of primary carboxylic acid amides, is known to exhibit a broad spectrum of biological activity including antimicrobial, antioxidant, anti-inflammatory, tranquiliser and antagonist properties (Jayadevappa, Nagendrappa et al. 2012). Metronidazole, a commonly used antibiotic in IBD, is metabolised to acetamide, mediated by the gut flora (Koch, Chrystal et al. 1979). In our study we see less urinary acetamide excreted post biological therapy. This may be due to the utilisation of metronidazole in pre-biological therapy patients.

4.9.3 Experiment 7 Summary

Metabolites in the carboxylic acids and derivatives class are increased in the urine of pre biological therapy IBD patients compared to post biological therapy IBD patients.

4.10 Experiment 8: Metabolite Identification Pre Biological v Post Biological Therapy (Paired)

In this experiment we interogate paired samples from the IBD patients undergoing biological therapy. The aim of using a paired analysis is to minimise background noise and to allow a direct comparison of a patient with themselves pre and post intervention (in this experiment the administration of anti TNFα therapy).

4.10.1 Experiment 8.1: Metabolite Identification Pre Biological v Post Biological Therapy (Paired) UHPLC-FTMS

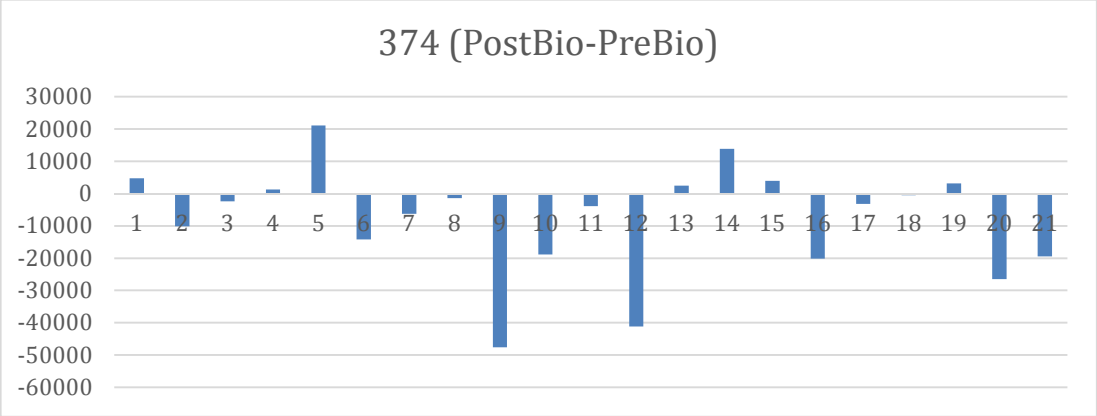
In the UHPLC-FTMS analyses of both serum and urine there were no significant metabolites identified that differentiated between paired patient samples pre- and post-biological therapy.

4.10.2 Experiment 8.2: Metabolite Identification Pre Biological v Post Biological Therapy (Paired) GC-ToF-MS

Table 4.34: Important Putative Metabolites Pre Biological Therapy v Post Biological Therapy (Paired) GC-ToF-MS

Variable ID	Biofluid	Quant mass	Retention time	Putative match	p value	q value
51	Urine	69	312.326	Unknown	0.011347	0.022694
374	Urine	321	1023.428	Lactose	0.021571	0.043142

Figure 4.61: Barchart Urinary Variable ID 374 Paired Sample Comparison of Post Biological Therapy to Pre Biological Therapy IBD patients



p=0.021571

In this experiment utilising paired samples, we can see that 14 patients have lower urinary lactose levels post biological therapy than pre-biological therapy, and 7 have higher lactose levels post biological therapy.

Lactose

Alpha-Lactose is the major sugar present in milk. Persons with lactose intolerance are unable to digest significant amounts of lactose. Common symptoms include abdominal pain and bloating, excessive flatus, and watery stool following the ingestion of foods containing lactose. Whilst lactose intolerance is common in IBD (Eadala, Matthews et al. 2011), this experiment utilises paired samples and therefore lactose intolerance would not become a factor. These findings are likely to be dietary in nature.

4.10.3 Experiment 8 Summary

In experiment 8, little differentiation is seen between the paired samples analysed. Using the patients as their own controls should minimise background noise and allow for an accurate representation of the change in metabolites related to disease state, and effect of treatment only. These results suggest that the metabolites identified in experiment 7 may simply represent background noise rather than truly significant findings.

4.11 Experiment 9: Metabolite Identification Pre Biological v Healthy Controls

In this experiment we aim to determine if differentiation between the group of pre biological therapy IBD patients and healthy controls is possible, in view of the small number of relevant metabolites identified in Experiments 7 and 8.

Table 4.35 Experiment 9 number of samples analysed

	Pre Biological	Healthy Controls
Serum samples	24	62
Urine samples	24	60

4.11.1 Experiment 9.1: Metabolite Identification Pre Biological v Healthy Controls UHPLC-FTMS

Table 4.36: Important Variables Identified Pre Biological Therapy v Healthy Controls UHPLC-FTMS

Variable ID	Biofluid	M/z	Retention Time	p value	q value
455	Urine	211.0567871	217.2532	0.00034964	0.01713236
544	Urine	237.1222813	180.762	0.0002237	0.0109613
726	Urine	295.1872401	709.07225	0.00097519	0.04778431
743	Urine	302.1587031	143.3935	0.0003303	0.0161847
784	Urine	322.075723	65.84785	0.00078133	0.03828517
800	Urine	334.0909909	299.601	3.74E-05	0.0018326

Table 4.36.1: Mass spectra search for 211.0567871 m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB32389	Methyl acrylate-divinylbenzene, completely hydrolyzed, copolymer	M+H+Na	211.056302	398.116109	0.0004851	Pyridines and derivatives
HMDB41102	Kanzonol O	M+H+K	211.056036	382.141638	0.0007511	Not classified
HMDB29515	Licoricone	M+H+K	211.056036	382.141638	0.0007511	Isoflavanoids
HMDB33148	Artocarpetin B	M+H+K	211.056036	382.141638	0.0007511	Flavanoids
HMDB33712	Glycyrin	M+H+K	211.056036	382.141638	0.0007511	Isoflavanoids
HMDB05000	Loratadine	M+H+K	211.05762	382.144806	0.0008329	Benzocyclohepatapyridines
HMDB59767	Tetrahydro-2,5-furan-diacetic	M+Na	211.057691	188.068473	0.0009039	Dicarboxylic acids and derivatives

	acid					
HMDB33300	(1RS,2RS)-Guaiacylglycerol 1-glucoside	M+2Na	211.057691	376.136947	0.0009039	Not classified
HMDB33301	(1x,2x)-Guaiacylglycerol 2-glucoside	M+2Na	211.057691	376.136947	0.0009039	Not classified
HMDB40600	(1x,2x)-Guaiacylglycerol 3-glucoside	M+2Na	211.057691	376.136947	0.0009039	Not classified

Table 4.36.2: Mass spectra search for 237.1222813 m/z

Compound	**Name**	**Adduct**	**Adduct MW (Da)**	**Compound MW (Da)**	**Delta**	**Class**
HMDB34671	(E)-5,8-Megastigmadien-4-one	M+2Na-H	237.122575	192.151415	0.0002937	Carbonyl compounds
HMDB36022	Isospirene	M+2Na-H	237.122575	192.151415	0.0002937	Dihydrofurans
HMDB29824	2,4-Diisopropyl-3-methylphenol	M+2Na-H	237.122575	192.151415	0.0002937	Benzene and substituted derivatives
HMDB35753	(R)-(E)-4,7-Megastigmadien-9-one	M+2Na-H	237.122575	192.151415	0.0002937	Prenol lipids
HMDB32541	4-(2,6,6-Trimethylcyclohex-1-enyl)but-2-en-4-one	M+2Na-H	237.122575	192.151415	0.0002937	Carbonyl compounds
HMDB36027	alpha-Damascone	M+2Na-H	237.122575	192.151415	0.0002937	Not classified
HMDB34959	Edulan I	M+2Na-H	237.122575	192.151415	0.0002937	Benzopyrans
HMDB29823	2,4-Diisopropyl-5-methylphenol	M+2Na-H	237.122575	192.151415	0.0002937	Prenol lipids
HMDB33545	(2E,4Z,7Z)-2,4,7-Tridecatrienal	M+2Na-H	237.122575	192.151415	0.0002937	Fatty acyls
HMDB29822	2,5-Diisopropyl-3-methylphenol	M+2Na-H	237.122575	192.151415	0.0002937	Prenol lipids

Table 4.36.3: Mass spectra search for 295.1872401 m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB60654	Citalopram N-oxide	M+2Na+H	295.187546	340.158706	0.0003059	Not classified
HMDB35918	7(14)-Bisabolene-2,3,10,11-tetrol	M+Na	295.187977	272.198759	0.0007369	Not classified
HMDB61157	N-(2-Hydroxyethyl)-morpholine N-oxide	2M+H	295.186363	147.089543	0.0008771	Oxazinanes
HMDB29449	(2R,3R,4R)-2-Amino-4-hydroxy-3-methylpentanoic acid	2M+H	295.186363	147.089543	0.0008771	Fatty acyls
HMDB33453	Fagomine	2M+H	295.186363	147.089543	0.0008771	Piperidines
HMDB41253	2,3-Diethylpyrazine	2M+Na	295.189315	136.100048	0.0020749	Diazines
HMDB36808	2,5-Diethylpyrazine	2M+Na	295.189315	136.100048	0.0020749	Diazines
HMDB14918	Phenelzine	2M+Na	295.189315	136.100048	0.0020749	Benzene and substituted derivatives
HMDB15644	Betahistine	2M+Na	295.189315	136.100048	0.0020749	Amines
HMDB32276	2-Ethyl-3,(5 or 6)-dimethylpyrazine	2M+Na	295.189315	136.100048	0.0020749	Diazines

Table 4.36.4: Mass spectra search for 302.1587031 m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB41889	Etamiphylline	M+Na	302.158743	279.169525	0.0000399	Not available (Super Class Alkaloids and derivatives)
HMDB15385	Lisdexamfetamine	M+K	302.16292	263.199762	0.0042169	Carboxylic acids and derivatives
HMDB30459	Hordatine B	M+H+Na	302.15433	580.312166	0.0043731	2-arylbenzofuran flavonoids

HMDB15237	Sibutramine	M+Na	302.164596	279.175378	0.0058929	Benzene and substituted derivatives
HMDB14339	Tramadol	M+K	302.151687	263.188529	0.0070161	Benzene and substituted derivatives
HMDB15646	Desvenlafaxine	M+K	302.151687	263.188529	0.0070161	Not classified
HMDB60532	O-Desmethylvenlafaxine	M+K	302.151687	263.188529	0.0070161	Not classified
HMDB29567	Hydroxy-alpha-sanshool	M+K	302.151687	263.188529	0.0070161	Alcohols and polyols
HMDB13892	N-Desmethylvenlafaxine	M+K	302.151687	263.188529	0.0070161	Not classified
HMDB15273	Doxepin	M+Na	302.151532	279.162314	0.0071711	Benzoxepines

Table 4.36.5: Mass spectra search for 322.075723 m/z

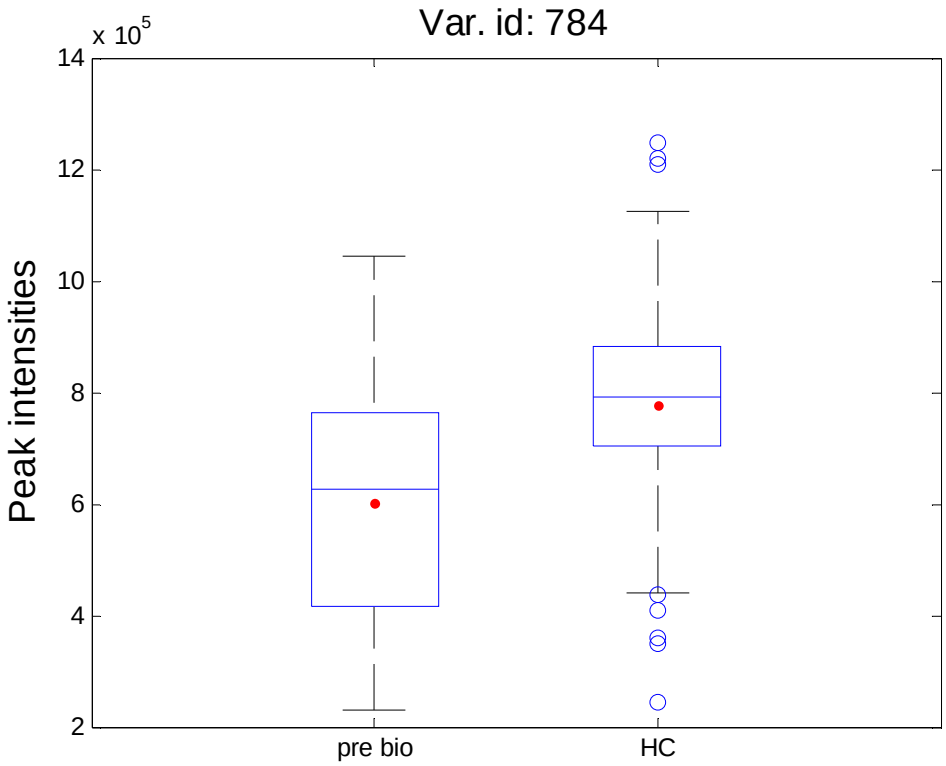
Compound	**Name**	**Adduct**	**Adduct MW (Da)**	**Compound MW (Da)**	**Delta**	**Class**
HMDB02044	8-Hydroxyguanosine	M+Na	322.075801	299.086583	0.000078	Purine nucleosides
HMDB41818	7-Aminoflunitrazepam	M+K	322.075248	283.11209	0.000475	Benzodiazepines
HMDB13220	Beta-Citryl-L-glutamic acid	M+H	322.076872	321.069596	0.001149	Carboxylic acids and derivatives
HMDB12947	Ferrocytochrome	M+H+K	322.073866	604.177298	0.001857	Not classified
HMDB29177	3'-O-Methyl-(-)-epicatechin-7-O-sulphate	M+2Na+H	322.077603	367.048763	0.00188	Flavanoids
HMDB61336	Teniposide catechol derivative	M+2H	322.077632	642.140712	0.001909	Lignan lactones
HMDB61280	Tenofovir Monophosphate	M+2Na+H	322.073511	367.044671	0.002212	Imidazopyimidines
HMDB37850	Myricetin 3,3'-digalactoside	M+2H	322.078883	642.143214	0.00316	Not classified
HMDB60815	Desmethylfrovatriptan	M+2K+H	322.071852	245.152812	0.003871	Indoles and derivatives
HMDB14618	Chlordiazepoxide	M+Na	322.071758	299.08254	0.003965	Benzodiazepines

Table 4.36.6: Mass spectra search for 334.0909909 m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB30704	Taxiphyllin	M+Na	334.08972	311.100502	0.0012709	Carbohydrates and carbohydrate conjugates
HMDB60471	Dhurrin	M+Na	334.08972	311.100502	0.0012709	Carbohydrates and carbohydrate conjugates
HMDB37841	N-(1-Deoxy-1-fructosyl)methionine	M+Na	334.093091	311.103873	0.0021001	Carbohydrates and carbohydrate conjugates
HMDB15585	Chlophedianol	M+2Na-H	334.094502	289.123342	0.0035111	Benzene and substituted derivatives
HMDB60463	Citalopram propionic acid	M+Na	334.08499	311.095772	0.0060009	Not classified
HMDB14045	3-Methoxymorphinan	M+2K+H	334.097004	257.177964	0.0060131	Morphinans
HMDB60552	Dextrorphan	M+2K+H	334.097004	257.177964	0.0060131	Morphinans
HMDB14992	Levorphanol	M+2K+H	334.097004	257.177964	0.0060131	Morphinans
HMDB39087	Sudachiin B	M+2H	334.097076	666.1796	0.0060851	Flavanoids
HMDB39088	Sudachiin C	M+2H	334.097076	666.1796	0.0060851	Not classified

None of the urinary metabolites identified in experiment 9.1 are biologically relevant in IBD.

Figure 4.62: Boxplot Urinary Variable ID 784 Pre Biological Therapy v Healthy Controls UHPLC-FTMS



p= 0.00078133

However, 8-hydroxyguanosine and myricetin 3,3'-digalactoside, identified under variable ID 784, may be of relevance in future biomarker and pathogenesis research.

8-hydroxyguanosine

8-hydroxyguanosine is a nucleoside that is an oxidative derivative of guanosine. It is a marker for measuring the rate of oxidative damage to nucleic acids and lipids. 8-hydroxydeoxyguanosine (8-OHdG) is formed when deoxyguanosine is oxidatively modified by reactive oxygen species. Although 8-hydroxyguanosine has never been considered as a biomarker in IBD, 8-OHdG has been (Thorsteinsdottir, Gudjonsson et al. 2011). The accumulation of 8-OHdG in colonic epithelial cells has been shown to increase with the duration of ulcerative colitis and the development of dysplasia. High levels of 8-OHdG are also found in ulcerative colitis-associated neoplasia. The major disadvantage of using 8-OHdG as a biomarker is that deoxyguanosine is rapidly oxidised to 8-OHdG during sample preparation and analysis, leading to the formation of artifacts, which can interfere with the results and thus it has not been established as a biomarker as yet.

Myricetin 3,3'-digalactoside

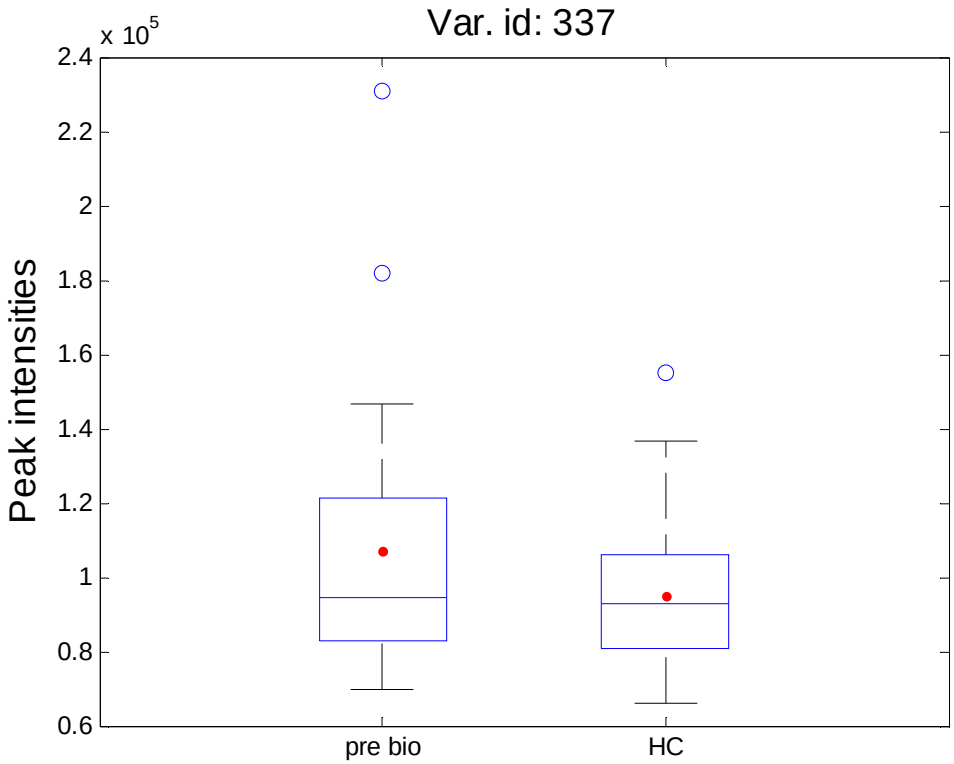
Myricetin 3,3'-digalactoside, a flavanoid, is found in herbs and spices. Flavanoids are known to have both anti-oxidant, anti-inflammatory, anti-carcinogenic and analgesia properties. Myricetin administered orally to mice with DSS induced colitis has been shown to reduced weight loss and reduce severity of colitis. It decreases the production of nitric oxide, myeloperoxidase and malondialdehyde, whilst increasing the activity of superoxide dismutase and glutathione peroxidise, as well as significantly reducing IL-1β and IL-6 levels (Zhao, Hong et al. 2013). Further investigation is required into its mechanisms of action but it may prove a useful therapy in IBD management, and interestingly in our study we see lower levels in pre biological therapy IBD patients than in HCs.

4.11.2 Experiment 9.2: Metabolite Identification Pre Biological v Healthy Controls GC-ToF-MS

Table 4.37: Important Putative Metabolites Pre Biological Therapy v Healthy Controls GC-ToF-MS

Variable ID	Biofluid	Quant mass	Retention time	Putative match	p value	q value
1109	Serum	368	954.378	Unknown	0.00016476	0.03641196
1113	Serum	369	957.078	Unknown	0.00015623	0.03452683
1146	Serum	382	954.428	Unknown	0.00017955	0.03968055
1344	Serum	441	954.628	Unknown	0.00010545	0.02330445
1347	Serum	442	954.478	Unknown	9.25E-05	0.0204425
1395	Serum	456	954.578	Unknown	0.00013387	0.02958527
1396	Serum	457	954.378	Unknown	0.00018582	0.04106622
337	Urine	259	786.278	Propanetric arboxylic acid	0.00091901	0.00551406
353	Urine	285	315.029	Glycine	0.0060148	0.0360888

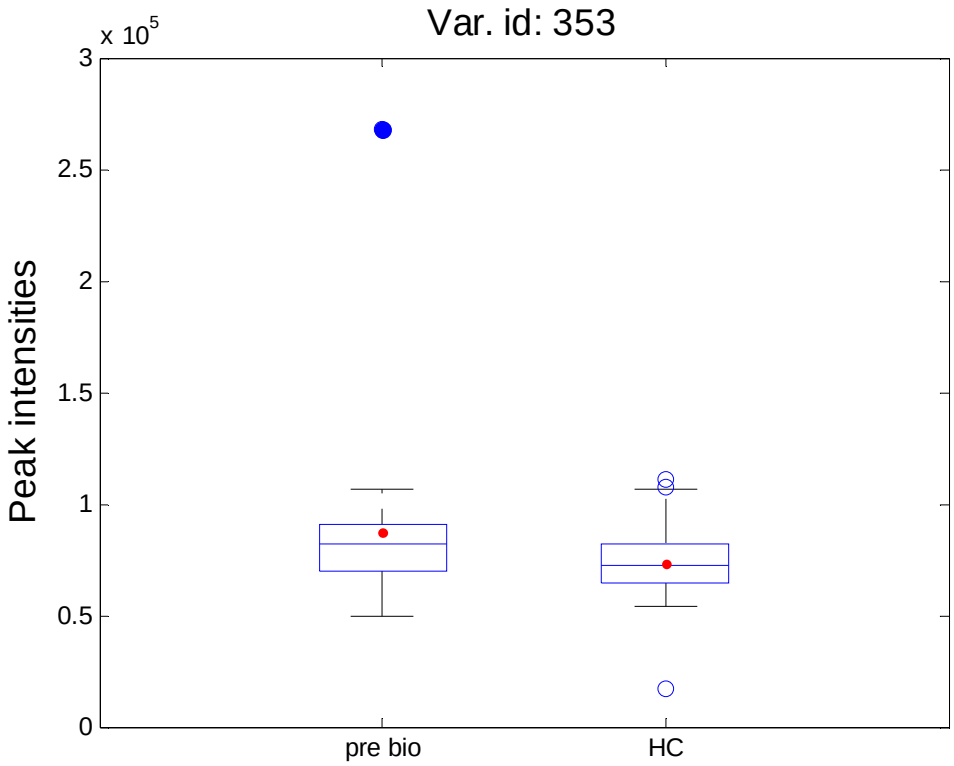
Figure 4.63: Boxplot Urinary Variable ID 337 Propanetricarboxylic acid Pre Biological Therapy v Healthy Controls GC-ToF-MS



p=0.00091901

Variable ID 337, identified as propanetricarboxylic acid from the carboxylic acid and derivatives class, has been discussed in detail in experiment 6.2. Here, urinary levels are seen to be higher in pre biological therapy IBD patients than in HCs. This may be due to increased secretion and thus lower serum levels as previously descrided.

Figure 4.64: Boxplot Urinary Variable ID 353 Glycine Pre Biological Therapy v Healthy Controls GC-ToF-MS



p=0.0060148

Glycine

Glycine is a simple amino acid belonging to the metabolite class of carboxylic acids and derviatives. It is synthesised from serine, threonine, choline, and hydroxyproline via inter-organ metabolism involving primarily the liver and kidneys. It is degraded through 3 pathways: the glycine cleavage system (forming ammonia and CO_2), serine hydroxymethytransferase, and conversion to glyoxylate by perioxisomal D-amino acid oxidase. Glycine is utilised for the biosynthesis of glutathione, heme, creatine, nucleic acids and uric acid. It is also a significant component of bile acid secretions and plays an important role in metabolic regulation, anti-oxidative reactions, and neurological function. Glycine has been shown to inhibit the production of proinflammatory cytokines (TNF-α, IL-1b and IL-6) and enhance IL-10. It can also exert anti-inflammatory effects during endothelial inflammation through inhibiting activation of NF-κB, degradation of inhibitor κBα, and expression of E-selectin. Therefore, this nutrient has been used to prevent tissue injury, enhance anti-oxidative capacity, promote protein synthesis and wound healing, improve immunity, and treat metabolic disorders in obesity, diabetes, cardiovascular disease, ischaemia-reperfusion injuries, cancers and inflammatory diseases (Wang, Wu et al. 2013). In our study we see higher levels of urinary glycine in pre biological therapy IBD patients than in HCs. Levels of glycine in IBD have been not been studied. We might expect to see increased

glycine levels in the serum of patients with an ongoing inflammatory process, as part of the inhibition of proinflammatory cytokine described above, or reduced levels allowing an uninhibited inflammatory process to continue. This requires further evaluation.

4.11.3 Experiment 9 Summary

Metabolites in the carboxylic acids and derivatives class are increased in the urine of pre biological therapy IBD patients compared to healthy controls.

4.12 Experiment 10: Metabolite Identification Post Biological v Healthy Controls

In this experiment we aim to determine whether differentiation between the group of post biological therapy IBD patients and healthy controls is possible.

Table 4.38 Experiment 10 number of samples analysed

	Post Biological	**Healthy Controls**
Serum samples	23	62
Urine samples	22	60

4.12.1 Experiment 10.1: Metabolite Identification Post Biological v Healthy Controls UHPLC-FTMS

Table 4.39: Important Variables Identified Post Biological v Healthy Controls UHPLC-FTMS

Variable ID	**Biofluid**	**M/z**	**Retention Time**	**p value**	**q value**
75	Urine	130.0491589	291.87825	0.00083544	0.0334176
590	Urine	250.0923999	110.4985	0.00036714	0.0073428
710	Urine	288.1252366	524.0685	0.00071216	0.0284864

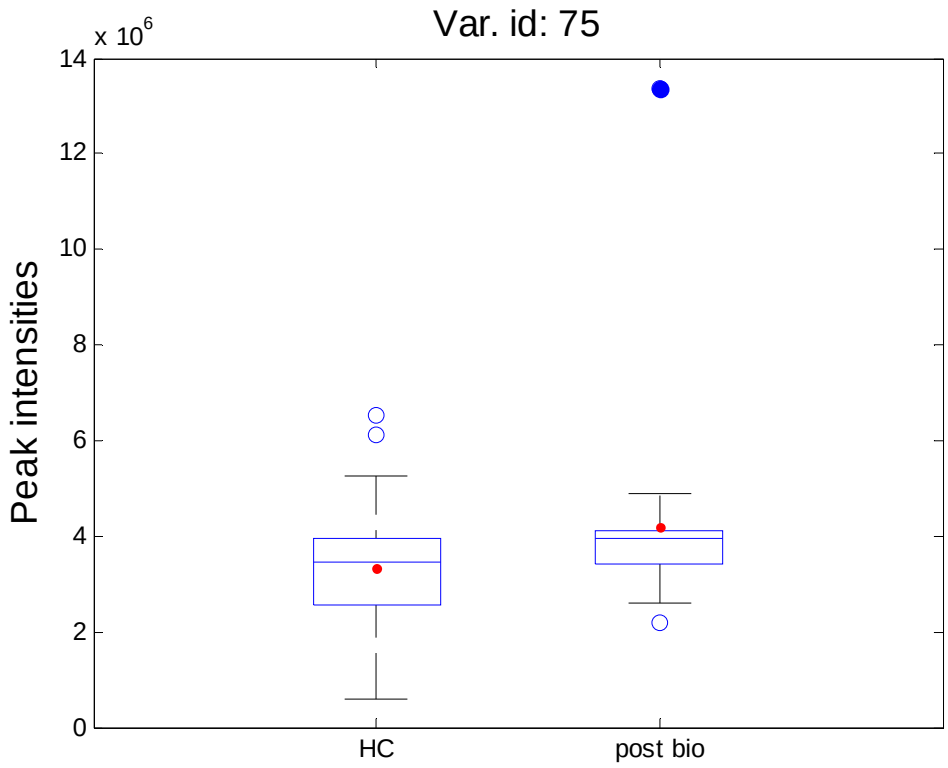
Table 4.39.1: Mass spectra search for 130.0491589 m/z

Compound	**Name**	**Adduct**	**Adduct MW (Da)**	**Compound MW (Da)**	**Delta**	**Class**
HMDB06239	S-aminomethyldihydrolipoamide	M+H+Na	130.049099	236.101705	0.0000599	Fatty acyls
HMDB28989	Phenylalanyl-Arginine	M+3Na	130.049248	321.18009	0.0000891	Not classified
HMDB28716	Arginyl-Phenylalanine	M+3Na	130.049248	321.18009	0.0000891	Carboxylic acids and derivatives
HMDB30986	2-Carboxy-5,7-dimethyl-4-octanolide	M+2Na	130.049473	214.120509	0.0003141	Lactones
HMDB30985	alpha-Carboxy-delta-decalactone	M+2Na	130.049473	214.120509	0.0003141	Lactones
HMDB00805	Pyrrolidonecarboxylic acid	M+H	130.049869	129.042593	0.0007101	Carboxylic acids and derivatives
HMDB01843	N-Acryloylglycine	M+H	130.049869	129.042593	0.0007101	Carboxylic acids and derivatives
HMDB61093	dimethadione	M+H	130.049869	129.042593	0.0007101	Azolines
HMDB023	Imidazoleac	M+2H	130.049869	258.085186	0.0007101	Imidazole

31	etic acid riboside					ribonucleosides and ribonucleotides
HMDB00267	Pyroglutamic acid	M+H	130.049869	129.042593	0.0007101	Carboxylic acids and derivatives

The variable ID 75 is shown to be higher in the urine of post biological therapy patients compared to healthy controls. The following metabolites may be of clinical relevance to IBD.

Figure 4.65: Boxplot Urinary Variable ID 75 Post Biological v Healthy Controls UHPLC-FTMS



p=0.00083544

S-aminomethyldihydrolipoamide

S-aminomethyldihydrolipoamide, belonging to the class of organic compounds known as fatty acyls, is an intermediate in the transfer of a methylamine group from gylcine via the glycine cleavage system. The enzyme glycine dehydrogenase catalyses the production, and consumption, of S-aminomethyldihydrolipoamide in the mitochondria. Interestingly we have previously see higher urinary levels of glycine in pre biological therapy IBD patient than in healthy controls, and this result may collaborate that finding, although the significance remains unclear.

Phenylalanyl-Arginine and Arginyl-Phenylalanine

Phenylalanyl-Arginine and Arginyl-Phenylalanine are dipeptides composed of phenylalanine and arginine. It is an incomplete breakdown product of protein digestion or protein catabolism.
Serum levels of phenylalanine have been identified in UC in both human (Zhang, Lin et al. 2013) and murine (Schicho, Nazyrova et al. 2010) models although this is as yet unexplained.
Serum levels of L-arginine are increased in murine DSS induced colitis, and dietary supplementation is associated with improvement in clinincal measures, reduced colonic permeability and number of myeloperoxidase-positive neutrophils and reduced proinflammatory cytokine and chemokine expression (Coburn, Gong et al. 2012). This would be in keeping with the findings in our study.

Pyrrolidonecarboxylic acid and pyroglutamic acid

Pyrrolidonecarboxylic acid and pyroglutamic acid are cyclic derivatives of glutamic acid, and belong to the class of organic compounds known as alpha amino acids and derivatives. Poly-γ-glutamic acid has been shown to attenuate weight loss, disease activity index and colonic shortening in DSS induced murine colitis, as well as reduce vascular endothelial growth factor-A and vascular endothelial growth factor receptor 2 expression and recruitment of leukocytes to the inflamed colon (Davaatseren, Hwang et al. 2013). Increased levels in the urine of post biological therapy patients may relate to a response to treatment.

Imidazoleacetic acid riboside

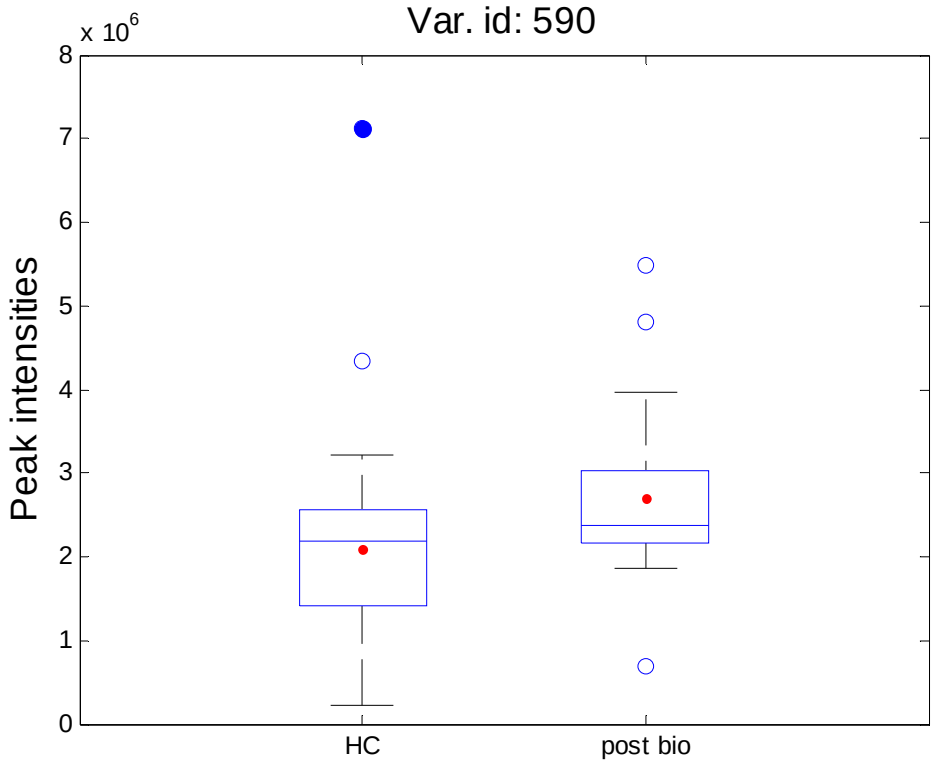
Imidazoleacetic acid riboside is a metabolite of imidazoleacetic acid. In kidney glomeruli, histamine is predominantly catabolised to acid metabolites of the diamine oxidase (histaminase) pathway, imidazoleacetic acid and imidazoleacetic acid riboside. In a murine model, histamine has been shown to have a proinflammatory role via histamine H4 receptor (Schirmer, Rezniczek et al. 2015), however a recent study in CD and UC in humans revealed no difference in histamine levels between IBD patients and healthy controls (Hagel, de Rossi et al. 2015). It is difficult to interpret our results in light of this finding.

Table 4.39.2: Mass spectra search for 250.0923999m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB35635	Kanokoside A	M+H+Na	250.092935	476.189376	0.0005351	Not classified
HMDB06878	S-Acetyldihydrolipoamide-E	M+H	250.092996	249.08572	0.0005961	Fatty acyls
HMDB01526	S-Acetyldihydrolipoamide	M+H	250.092996	249.08572	0.0005961	Fatty acyls
HMDB39214	Butyl (S)-3-hydroxybutyrate [arabinosyl-(1->6)-glucoside]	M+2Na	250.091731	454.205027	0.0006689	Not classified
HMDB138	N4-	M+2Na+	250.0915	295.0626	0.00088	Benzene

54	Acetylsulfamethoxazole	H	17	77	29	and substituted derivatives
HMDB30495	Artonol D	M+2H	250.091203	498.167853	0.0011969	Not classified
HMDB36742	Eucaglobulin	M+2H	250.094139	498.173726	0.0017391	Saccharolipids
HMDB38681	Musabalbisiane B	M+2H	250.094139	498.173726	0.0017391	Prenol lipids
HMDB39249	1-(2H-1,3-Benzodioxol-5-yl)-2-[2,6-dimethoxy-4-(prop-2-en-1-yl)phenoxy]propyl benzoate	M+H+Na	250.089999	476.183503	0.0024009	Not classified
HMDB61254	Trabectedin metabolite M8b (ET-729)	M+3H	250.089336	747.24618	0.0030639	Benzene and substituted derivatives

Figure 4.66: Boxplot Urinary Variable ID 590 Post Biological v Healthy Controls UHPLC-FTMS



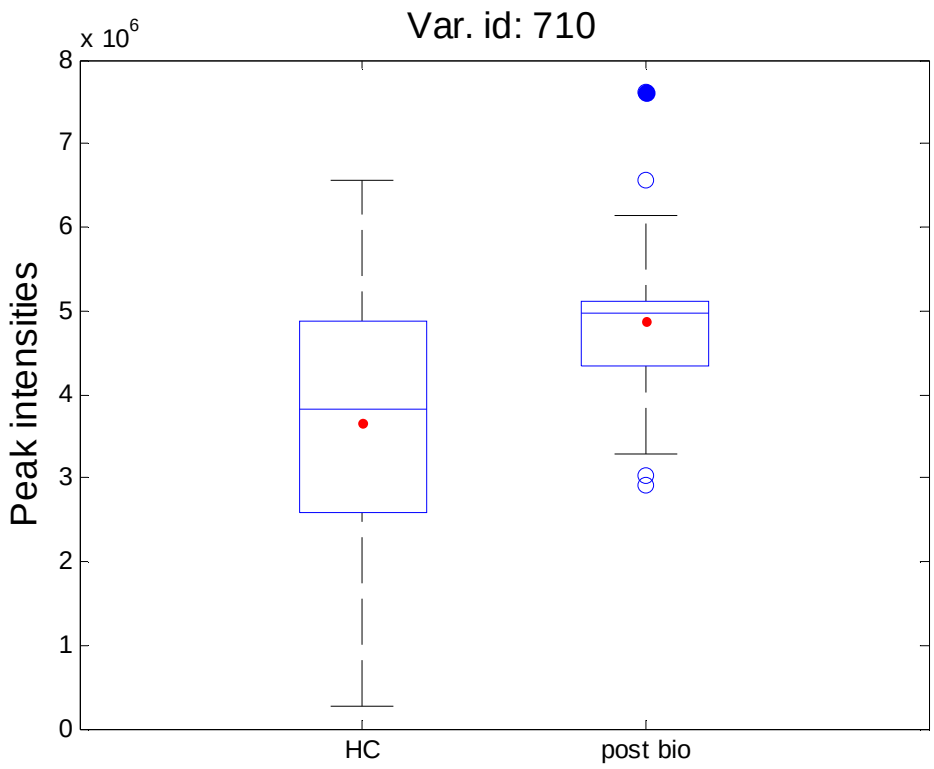
p=0.00036714

None of the urinary metabolites identified have any relation to IBD. They are all dietary or medication related, and the medication related metabolites may account for higher urinary levels seen in post biological therapy IBD patients.

Table 4.39.3: Mass spectra search for 288.1252366 m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB34679	Cinncassiol D2 glucoside	M+2Na	288.125574	530.272712	0.0003374	Prenol lipids
HMDB33486	Pipermethystine	M+H	288.123034	287.115758	0.0022026	Benzene and substituted derivatives
HMDB12486	(R,S)-Norlaudanosoline	M+H	288.123034	287.115758	0.0022026	Not classified
HMDB61073	Noroxymorphone	M+H	288.123034	287.115758	0.0022026	Morphinans
HMDB34075	Austalide C	M+2H	288.127982	574.241412	0.0027454	Benzopyrans
HMDB29022	Prolyl-Lysine	M+2Na-H	288.129452	243.158292	0.0042154	Not classified
HMDB28959	Lysyl-Proline	M+2Na-H	288.129452	243.158292	0.0042154	Carboxylic acids and derivatives
HMDB15254	Ambenonium	M+H+K	288.119458	536.268482	0.0057786	Carboxylic acids and derivatives
HMDB61015	*Ortho*-hydroxyatorvastatin	M+2H	288.131234	574.247915	0.0059974	Not classified
HMDB61014	*Para*-hydroxyatorvastatin	M+2H	288.131234	574.247915	0.0059974	Not classified

Figure 4.67: Boxplot Urinary Variable ID 710 Post Biological v Healthy Controls UHPLC-FTMS



p=0.00071216

The variable ID 710 is shown to be higher in the urine of post biological therapy patients compared to healthy controls. This is likely to be accounted for by medications.

Ortho-hydroxyatorvastatin and _Para_-hydroxyatorvastatin

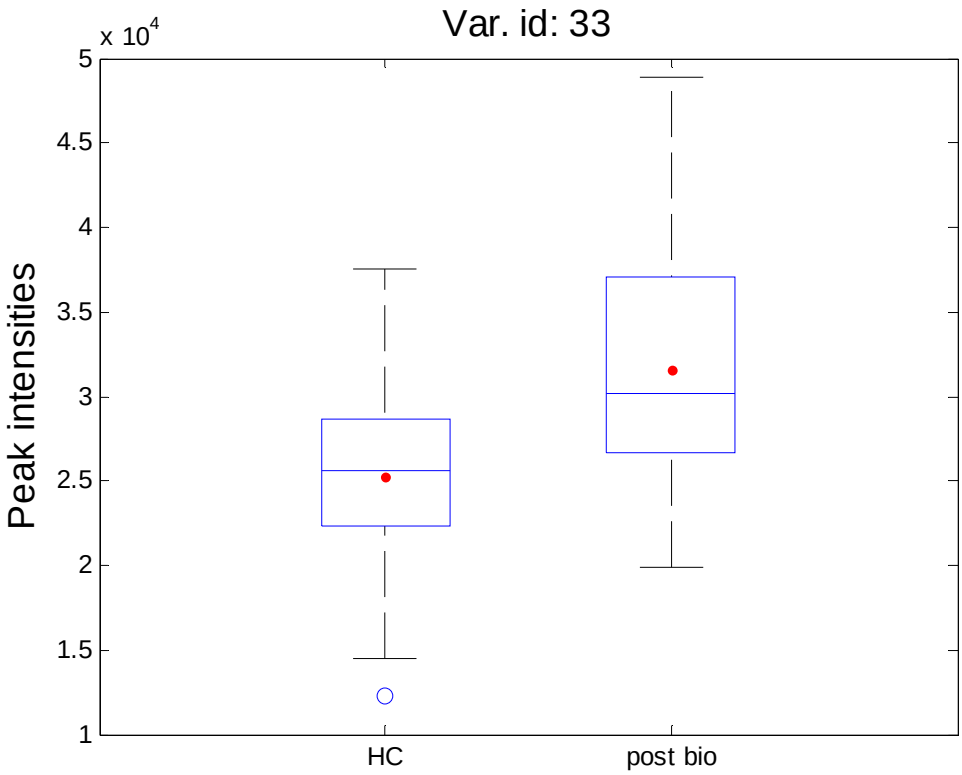
Ortho-hydroxyatorvastatin and *Para*-hydroxyatorvastatin are metabolites of atorvastatin. Statins are known to reduce chemokine expression in coronary artery disease. CXCL10 is a ligand for the CXCR3 receptor, the activation of which results in the recruitment of T lymphocytes and the perpetuation of mucosal inflammation. Atorvastatin has been shown to reduce plasma CXCL10 levels in CD patients (Grip, Janciauskiene 2009) but is not currently a standard therapy.

4.12.2 Experiment 10.2: Metabolite Identification Post Biological v Healthy Controls GC-ToF-MS

Table 4.40: Putative Metabolites Post Biological Therapy v Healthy Controls GC-ToF-MS

Variable ID	Biofluid	Quant mass	Retention time	Putative match	p value	q value
2	Serum	45	354.576	Unknown	9.93E-06	0.00303858
21	Serum	51	355.301	Unknown	2.09E-05	0.0063954
26	Serum	52	355.584	Unknown	8.42E-06	0.00257652
33	Serum	53	353.773	Isotridecanol	6.55E-05	0.020043
58	Serum	59	354.938	Unknown	1.03E-05	0.0031518
80	Serum	66	355.789	Unknown	3.29E-05	0.0100674
87	Serum	67	354.848	Unknown	3.89E-05	0.0119034
109	Serum	72	355.624	Unknown	2.73E-05	0.0083538
112	Serum	73	355.538	Unknown	4.11E-05	0.0125766
169	Serum	87	355.848	Unknown	2.12E-05	0.0064872
171	Serum	88	355.468	Unknown	2.96E-05	0.0090576
177	Serum	89	355.368	Unknown	8.77E-06	0.00268362
198	Serum	94	357.848	Unknown	4.70E-05	0.014382
226	Serum	101	356.236	Unknown	6.18E-06	0.00189108
283	Serum	115	356.848	Unknown	4.30E-05	0.013158
290	Serum	117	355.488	Unknown	3.15E-05	0.009639
293	Serum	118	355.451	Unknown	2.79E-05	0.0085374
344	Serum	129	355.924	Unknown	1.90E-05	0.005814
352	Serum	131	356.183	Unknown	1.40E-05	0.004284
360	Serum	133	355.593	Unknown	2.46E-05	0.0075276
367	Serum	135	355.638	Unknown	1.99E-05	0.0060894
417	Serum	147	355.621	Unknown	3.00E-05	0.00918
420	Serum	148	355.588	Unknown	2.17E-05	0.0066402
423	Serum	149	355.524	Unknown	1.94E-05	0.0059364
522	Serum	175	355.768	Unknown	3.75E-05	0.011475
577	Serum	190	355.338	Unknown	2.20E-05	0.006732
581	Serum	191	355.326	Unknown	1.93E-05	0.0059058
585	Serum	192	355.298	Unknown	1.51E-05	0.0046206
590	Serum	193	356.103	Unknown	1.48E-05	0.0045288
635	Serum	203	355.328	Unknown	2.68E-05	0.0082008
690	Serum	219	355.371	Unknown	1.45E-05	0.004437
697	Serum	220	355.596	Unknown	1.54E-05	0.0047124
700	Serum	221	355.586	Unknown	1.22E-05	0.0037332
1113	Serum	369	957.078	Unknown	3.98E-05	0.0121788

Figure 4.68: Boxplot Serum Variable ID 33 Isotridecanol Post Biological Therapy v Healthy Controls GC-ToF-MS



p=6.55E-05

In this experiment, the only identified substance is isotridecanol, a fatty alcohol. No relation to inflammation or IBD has been identified. We see higher levels in the post biological therapy group than the healthy controls, but this is unexplained.

4.12.3 Experiment 10 Summary

Metabolites in the carboxylic acids and derivatives class, the fatty acyls class, and the imidazole ribonucleosides and ribonucleotides class are increased in the urine of post biological therapy IBD patients compared to healthy controls. Metabolites in the fatty alcohols class are increased in the serum of post biological therapy IBD patients compared to healthy controls.

4.13 Experiment 11: Metabolite Identification Pre Surgery v Post Surgery (Grouped)

In experiment 11 we consider the IBD patients undergoing surgery, and aim to determine whether differentiation between the pre and post surgery groups is possible. In this experiment the samples are grouped as pre and post surgery. Future experiments will consider paired samples.

Table 4.41 Experiment 11 number of samples analysed

	Pre-surgery	**Post-surgery**
Serum samples	30	28
Urine samples	30	28

4.13.1 Experiment 11.1: Metabolite Identification Pre Surgery v Post Surgery (Grouped) UHPLC-FTMS

Table 4.42: Important Variables Identified Pre Surgery v Post Surgery (Grouped) UHPLC-FTMS

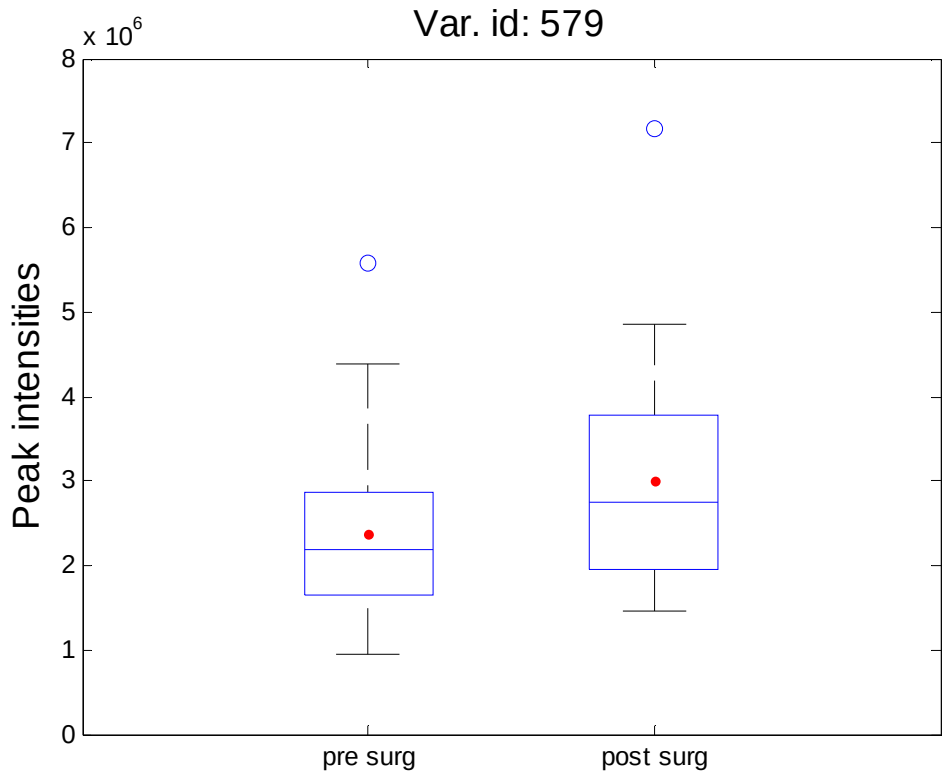
Variable ID	**Biofluid**	**M/z**	**Retention time**	**p value**	**q value**
579	Serum	425.3168199	1033.3427	0.0045059	0.0360472

Table 4.42.1: Mass spectra search for 425.3168199 m/z

Compound	**Name**	**Adduct**	**Adduct MW (Da)**	**Compound MW (Da)**	**Delta**	**Class**
HMDB00067	Cholesterol	M+K	425.318024	386.354866	0.0012041	Steroids and steroid derivatives
HMDB06841	5a-Cholest-8-en-3b-ol	M+K	425.318024	386.354866	0.0012041	Steroids and steroid derivatives
HMDB59604	5-beta-Cholestan-3-one	M+K	425.318024	386.354866	0.0012041	Steroids and steroid derivatives
HMDB00871	5alpha-Cholestanone	M+K	425.318024	386.354866	0.0012041	Steroids and steroid derivatives
HMDB11182	5beta-Cholestanone	M+K	425.318024	386.354866	0.0012041	Steroids and steroid derivatives
HMDB01170	Lathosterol	M+K	425.318024	386.354866	0.0012041	Steroids and steroid derivatives
HMDB29815	Doristerol	M+K	425.318024	386.354866	0.0012041	Steroids and steroid derivatives
HMDB29340	Ceanothine C	M+2Na+H	425.318146	470.289306	0.0013261	Carboxylic acids and derivatives
HMDB59902	2,6-Diisopropyl	2M+H	425.320277	212.156501	0.0034571	Naphthalenes

	naphthalene					
HMDB60336	1,4'-Bipiperidine-1'-carboxylic acid	2M+H	425.312232	212.152478	0.0045879	Piperidines

Figure 4.69: Boxplot Serum Variable ID 579 Pre Surgery v Post Surgery (Grouped) UHPLC-FTMS



p=0.0045059

In this experiment, peak intensities of serum variable ID 579 are higher in the post surgery group than in the pre-surgery group. Cholesterol, 5-beta-cholestan-3-one and lathosterol are considered relevant.

Cholesterol

Cholesterol, belonging to the class of organic compounds known as steroids and steroid derivatives, is a sterol (a combination steroid and alcohol) and a lipid found in the cell membranes of all body tissues, and transported in the blood plasma of all animals. IBD patients exhibit lower levels of low-density lipoprotein cholesterol and total cholesterol than healthy controls, with this effect being more profound in CD than UC. These findings are independent of disease activity and are also observed post operatively (Agouridis, Elisaf et al. 2011). In our study, cholesterol levels are seen to be lower in the pre-operative group than the post-operative group. This may be due to malabsorption in the pre-

operative group or the presence of pro-inflammatory cytokines and autoantibodies against lipoprotein lipase altering lipoprotein metabolism (Sappati Biyyani, Putka et al. 2010).

5-beta-cholestan-3-one

5-beta-cholestan-3-one is part of the primary bile acid biosynthesis and steroid hormone biosynthesis pathways. It belongs to the class of organic compounds known as cholesterols and derivatives. It has been postulated that IBD-associated dysbiosis leads to modifications in bile acid pool composition and can affect gut homeostasis (Duboc, Rajca et al. 2013). Bile acids are known to be anti-inflammatory molecules able to decrease the synthesis of proinflammatory cytokines such as TNF-α in monocytes and macrophages through NF-κB inhibition. In our study we see lower levels of 5-beta-cholestan-3-one in pre-operative patients compared to post-operative patients. This may alter bile acid composition and thus start the cycle of bile acid dysmetabolism, and lack of anti-inflammatory effect. In post-operative patients it may be that we are seeing more normal levels of 5-beta-cholestan-3-one when gut homeostasis should be improved.

Lathosterol

Lathosterol is a sterol and a lipid found in the cell membranes of all body tissues, and transported in the blood plasma of all animals. It belongs to the class of organic compounds known as cholesterols and derivatives, and is used as an indicator of whole-body cholesterol synthesis. Plasma lathosterol levels are significantly lower in patients with active CD than in healthy controls (Agouridis, Elisaf et al. 2011). This finding is replicated in our pre- and post-operative analyses.

Lathosterol

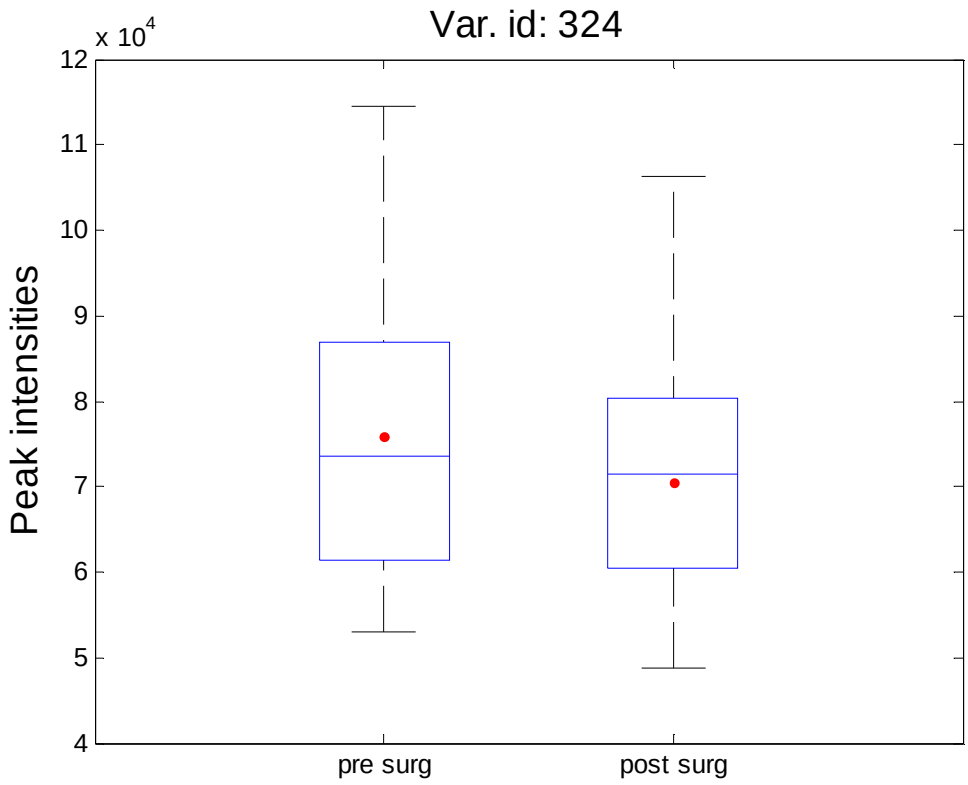
Lathosterol is a sterol and a lipid found in the cell membranes of all body tissues, and transported in the blood plasma of all animals. It belongs to the class of organic compounds known as cholesterols and derivatives, and is used as an indicator of whole-body cholesterol synthesis. Plasma lathosterol levels are significantly lower in patients with active CD than in healthy controls (Agouridis, Elisaf et al. 2011). This finding is replicated in our pre- and post-operative analyses.

4.13.2 Experiment 11.2: Metabolite Identification Pre Surgery v Post Surgery (Grouped) GC-ToF-MS

Table 4.43: Putative Metabolites Pre Surgery v Post Surgery (Grouped) GC-ToF-MS

Variable ID	Biofluid	Quant mass	Retention time	Putative match	p value	q value
1145	Serum	381	953.529	Unknown	0.00037018	0.03923908
324	Urine	233	593.988	Erythritol	0.0015129	0.0090774
337	Urine	259	786.278	Propanetricarboxylic acid	0.0080796	0.0484776

Figure 4.70: Boxplot Urinary Variable ID 324 Erythritol Pre Surgery v Post Surgery (Grouped) GC-ToF-MS



p=0.0015129

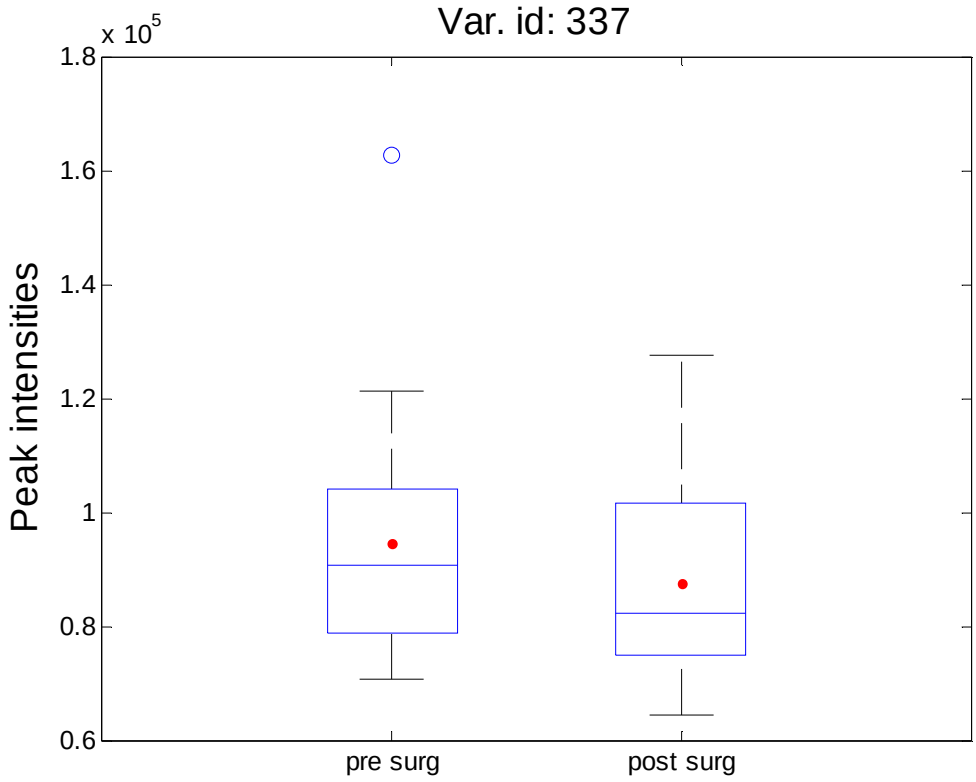
In this experiment, higher peak intensities of variable ID 324, erythritol, are seen in the pre-operative group than in the post-operative group.

Erythritol

Erythritol belongs to the class of organic compounds known as sugar alcohols. It is used as a food additive and occurs naturally in some fruit and fermented foods. In our study we see higher urinary

levels in pre-operative compared to post-operative patients, however, no links with IBD have been identified and this result is unexplained but may purely be dietary.

Figure 4.71: Boxplot Urinary Variable ID 337 Propanetricarboxylic acid Pre Surgery v Post Surgery (Grouped) GC-ToF-MS



p=0.0080796

In this experiment higher peak intensities of variable ID 337, propanetricarboxylic acid, are seen in the pre-operative group than in the post-operative group.

Propanetricarboxylic acid

Propanetricarboxylic acid has previously been described in detail in experiment 6.2. In this experiment we see higher levels of propanetricarboxylic acid in the urine of the pre-operative group compared to the post-operative group. This may mean that pre-operatively less TCA cycle-related downstream molecules are available due to inhibition of aconitase, and post-operatively less inhibition results in a normalisation of ATP production.

4.13.3 Experiment 11 Summary

Metabolites in the class steroids and steroid derivatives are increased in the serum of post surgery IBD patients compared to pre surgery IBD patients. Metabolites in the classes carbohydrates and carbohydrate conjugates, and carboxylic acids and derivatives are increased in the urine of pre surgery IBD patients compared to post surgery IBD patients.

4.14 Experiment 12: Metabolite Identification Pre Surgery v Post Surgery (Paired)

In this experiment we utilise paired samples in IBD patients undergoing surgery. This allows each patients to act as their own control pre and post intervention (in this case surgery).

Table 4.44 Experiment 12 number of samples analysed

	Pre-surgery	**Post-surgery**
Serum samples	30	28
Urine samples	30	28

4.14.1 Experiment 12.1: Metabolite Identification Pre Surgery v Post Surgery (Paired) UHPLC-FTMS

Table 4.45: Important Variables Identified Pre Surgery v Post Surgery (Paired) UHPLC-FTMS

Variable ID	**Biofluid**	**M/z**	**Retention time**	**p value**	**q value**
579	Serum	425.3168199	1033.3427	0.0090494	0.0361976
750	Urine	303.1256521	327.27085	0.0020453	0.040906

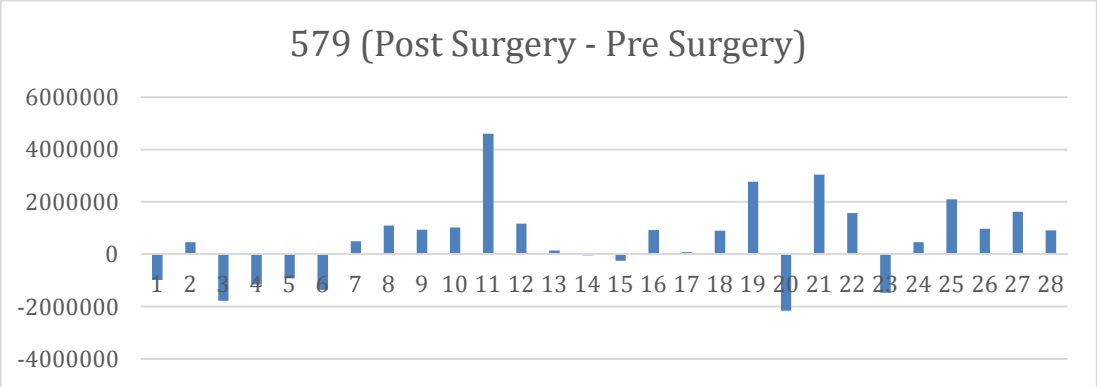
Table 4.45.1: Mass spectra search for 425.3168199 m/z

Compound	**Name**	**Adduct**	**Adduct MW (Da)**	**Compound MW (Da)**	**Delta**	**Class**
HMDB00067	Cholesterol	M+K	425.318024	386.354866	0.0012041	Steroids and steroid derivatives
HMDB06841	5a-Cholest-8-en-3b-ol	M+K	425.318024	386.354866	0.0012041	Steroids and steroid derivatives
HMDB59604	5-beta-Cholestan-3-one	M+K	425.318024	386.354866	0.0012041	Steroids and steroid derivatives
HMDB00871	5alpha-Cholestanone	M+K	425.318024	386.354866	0.0012041	Steroids and steroid derivatives
HMDB11182	5beta-Cholestanone	M+K	425.318024	386.354866	0.0012041	Steroids and steroid derivatives
HMDB01170	Lathosterol	M+K	425.318024	386.354866	0.0012041	Steroids and steroid derivatives
HMDB29815	Doristerol	M+K	425.318024	386.354866	0.0012041	Steroids and steroid derivatives
HMDB29340	Ceanothine C	M+2Na+H	425.318146	470.289306	0.0013261	Carboxylic acids and derivatives
HMDB59902	2,6-Diisopropylnaphthalene	2M+H	425.320277	212.156501	0.0034571	Naphthalenes

HMDB60336	1,4'-Bipiperidine-1'-carboxylic acid	2M+H	425.312232	212.152478	0.0045879	Piperidines

These metabolites have previously been discussed in experiment 11.1. Those of biological relevance are cholesterol, 5-beta-cholestan-3-one and lathosterol.

Figure 4.72: Barchart Serum Variable ID 579 paired sample comparison of post surgery to pre surgery IBD patients



p=0.0090494

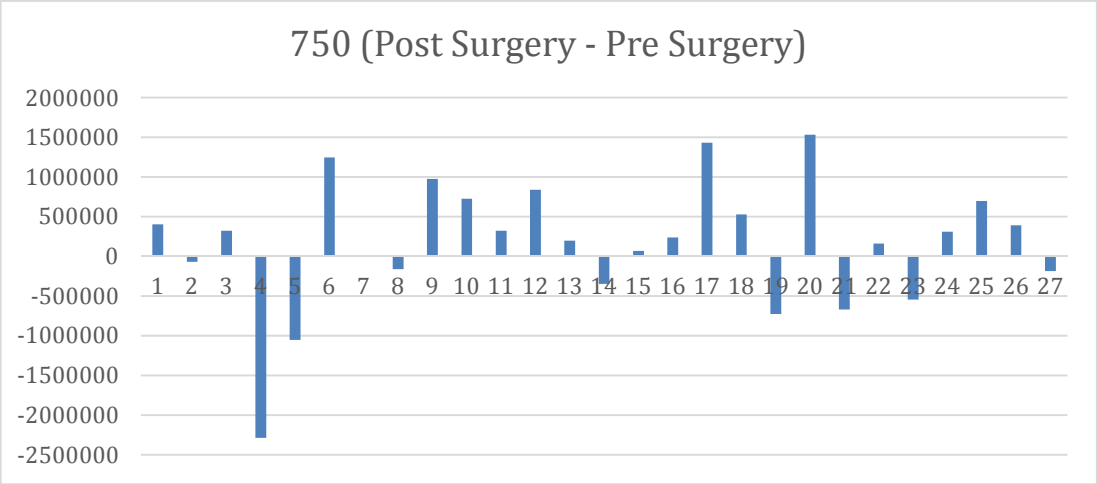
Variable ID 579 shows increased serum levels in post surgery patients compared to pre surgery patients in 19 of the 28 sample sets.

Table 4.45.2: Mass spectra search for 303.1256521 m/z

Compound	**Name**	**Adduct**	**Adduct MW (Da)**	**Compound MW (Da)**	**Delta**	**Class**
HMDB15620	Mianserin	M+K	303.125807	264.162649	0.0001549	Benzazepines
HMDB33189	Isomucronulatol	M+H	303.1227	302.115424	0.0029521	Not classified
HMDB33996	2',7-Dihydroxy-4',6-dimethoxyisoflavan	M+H	303.1227	302.115424	0.0029521	Isoflavanoids
HMDB41653	3'-Hydroxy-3,4,5,4'-tetramethoxystilbene	M+H	303.1227	302.115424	0.0029521	Stilbenes
HMDB30717	(R)-3',7-Dihydroxy-2',4'-dimethoxyisoflavan	M+H	303.1227	302.115424	0.0029521	Isoflavanoids
HMDB381	(±)-	M+H	303.1227	302.115424	0.0029521	Not

28	Sphaerosin					classified
HMDB01008	Biliverdin	M+H+Na	303.122164	582.247835	0.0034881	Tetrapyrroles and derivatives
HMDB29313	Canescein	M+H+K	303.121573	566.272712	0.0040791	Steroids and steroid derivatives
HMDB02379	Mesoporphyrin IX	M+H+K	303.12987	566.289306	0.0042179	Tetrapyrroles and derivatives
HMDB40236	3,4-Diethylthiophene	2M+Na	303.12116	140.065971	0.0044921	Heteroaromatic compounds

Figure 4.73: Barchart Urinary Variable ID 750 UHPLC-FTMS paired sample comparison of post surgery to pre surgery IBD patients



p=0.0020453

Variable ID 750 shows increased urinary levels in post surgery patients compared to pre surgery patients in 17 of the 27 sample sets. The following metabolites are potentially biologically relevant.

3'-Hydroxy-3,4,5,4'-tetramethoxystilbene

3'-Hydroxy-3,4,5,4'-tetramethoxystilbene, a polyphenol belonging to the stilbenes class of organic compounds, has no links with IBD. However, resveratrol (3,5,40-trihydroxy-trans-stilbene) has been shown to down-regulate inflammatory pathways of MAPK and NF-κB, lessen COX-2, modify cytokines, diminish leucocytes, alter intestinal microflora and decrease clinical symptoms in animal models of IBD (Farzaei, Rahimi et al. 2015).

Biliverdin

Biliverdin belongs to the class of organic compounds known as bilirubins and is formed as a byproduct of haemoglobin breakdown. Heme is catabolised by heme oxygenase into biliverdin, free iorn and carbon monoxide. It is then reduced to bilirubin by biliverdin reductase. Biliverdin is

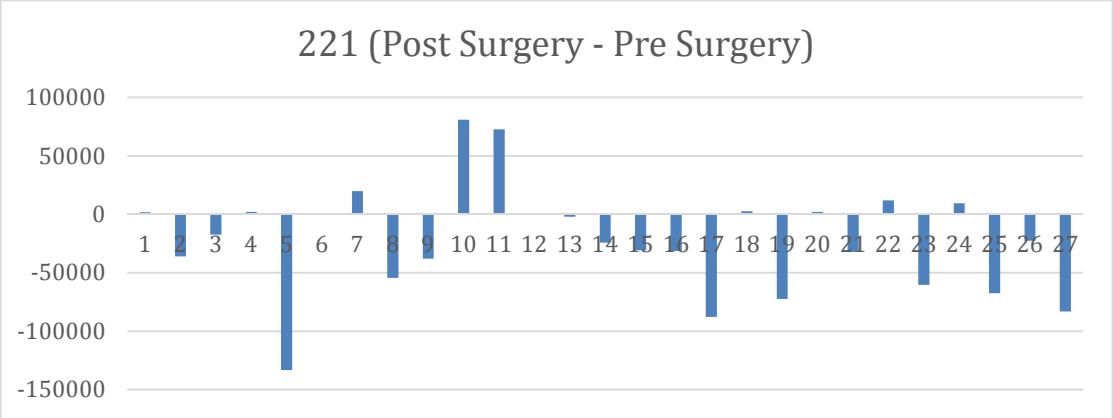
cytoprotective and has anti-inflammatory effects (Wegiel, Otterbein 2012). Heme oxygenase-1, an isoenzyme of heme oxygenase, is transcriptionally induced in response to oxidative stress and may play a protective role in intestinal damage. Both carbon monoxide and biliverdin have been implicated as mediators in this response (Naito, Takagi et al. 2004). More recently, heme oxygenase-1 and carbon monoxide may be novel therapeutic molecules in patients with IBD (Takagi, Naito et al. 2010, Takagi, Uchiyama et al. 2015).

4.14.2 Experiment 12.2: Metabolite Identification Pre Surgery v Post Surgery (Paired) GC-ToF-MS

Table 4.46: Putative Metabolites Pre Surgery v Post Surgery (Paired) GC-ToF-MS

Variable ID	Biofluid	Quant mass	Retention time	Putative match	p value	q value
221	Urine	164	755.278	Sorbitol	0.0035504	0.0213024
324	Urine	233	593.988	Erythritol	0.0020453	0.0122718

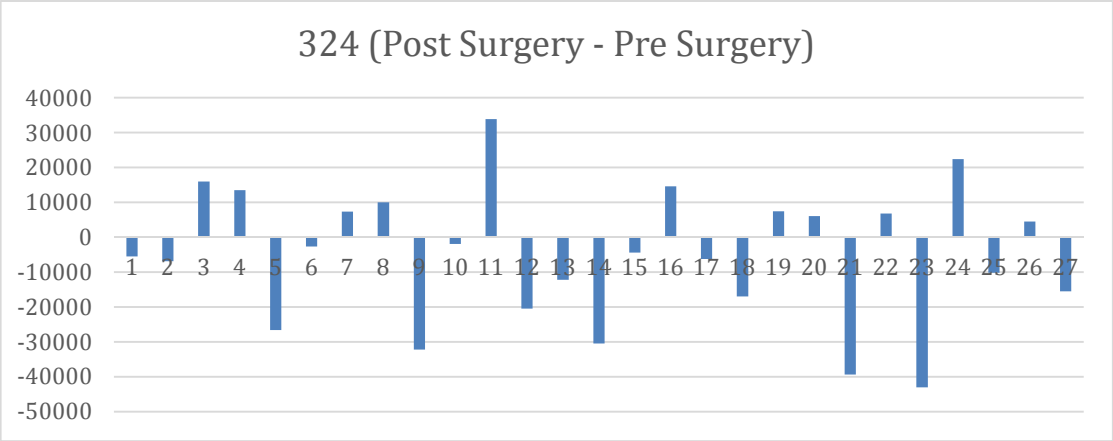
Figure 4.74: Barchart Urinary Variable ID 221 GC-ToF-MS paired sample comparison of post surgery to pre surgery IBD patients



p=0.0035504

Variable ID 221 identified as sorbitol, shows increased urinary levels in post surgery patients compared to pre surgery patients in 16 of the 27 sample sets.

Figure 4.75: Barchart Urinary Variable ID 324 GC-ToF-MS paired sample comparison of post surgery to pre surgery IBD patients



p=0.0020453

Variable ID 324 identified as erythritol, shows reduced urinary levels in post surgery patients compared to pre surgery patients in 15 of the 27 sample sets.

Neither of the urinary metabolites identified in this experiment are of biological relevance in IBD. Both, from the carbohydrates and carbohydrate conjugates class, are likely related to dietary intake.

4.14.3 Experiment 12 Summary

On the UHPLC-FTMS platform, cholesterol, 5-beta-cholestan-3-one and lathosterol appear to be increased in the serum of the majority of post-operative patients, compared to their pre-operative samples. These compounds all belong to the steroid and steroid derivatives class.

In the urine of post-operative patients compared to their pre-operative samples, 3'-Hydroxy-3,4,5,4'-tetramethoxystilbene, from the stilbenes class, and Biliverdin, from the tetrapyrroles and derivatives class, are biologically relevant metabolites that have increased levels in the majority of patients.

On the GS-ToF-MS platform, the metabolites identifed, sorbitol and erythritol from the carbohydrates and carbohydrate conjugates class, are not of biological relevance. However, sorbitol apprears to be increased and erythritol decreased in the urine of the majority of patients post surgery compared to when they are in the pre-operative period.

4.15 Experiment 13: Metabolite Identification Pre Surgery v Healthy Controls

Experiment 13 aims to determine whether differentiation between the profiles of pre surgery IBD patients, and HCs is possible.

Table 4.47 Experiment 13 number of samples analysed

	Pre-surgery	**Healthy Controls**
Serum samples	30	62
Urine samples	30	60

4.15.1 Experiment 13.1: Metabolite Identification Pre Surgery v Healthy Controls UHPLC-FTMS

Table 4.48: Important Variables Identified Pre Surgery v Healthy Controls UHPLC-FTMS

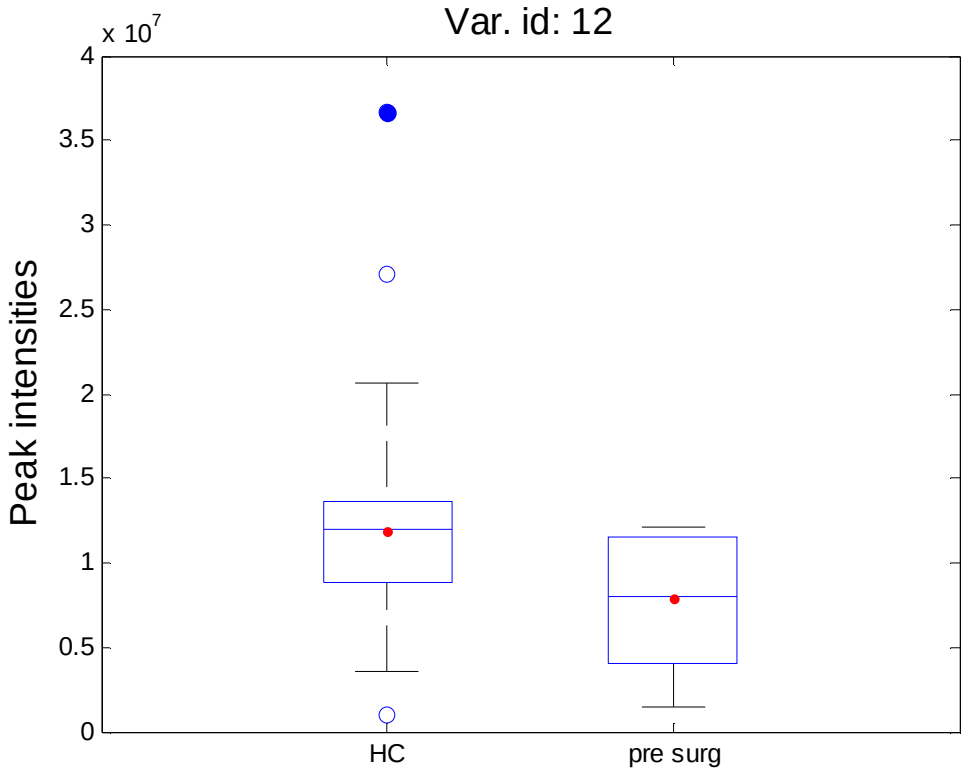
Variable ID	**Biofluid**	**M/z**	**Retention time**	**p value**	**q value**
12	Urine	106.0362854	240.0221	8.53E-07	4.69E-05
174	Urine	153.0727774	228.6984	0.00026143	0.01437865
258	Urine	169.1213976	500.2978	2.81E-05	0.0015455
407	Urine	200.1999862	697.53385	0.00067961	0.03737855
597	Urine	253.0654594	62.4078	0.00079279	0.04360345
610	Urine	257.1488092	361.4173	6.65E-05	0.0036575
743	Urine	302.1587031	143.3935	0.00040501	0.02227555
784	Urine	322.075723	65.84785	6.19E-05	0.0034045
800	Urine	334.0909909	299.601	1.02E-06	5.61E-05

Table 4.48.1: Mass spectra search for 106.0362854 m/z

Compound	**Name**	**Adduct**	**Adduct MW (Da)**	**Compound MW (Da)**	**Delta**	**Class**
HMDB15126	Oxaprozin	M+2H+Na	106.036321	293.105193	0.0000356	Azoles
HMDB33048	Ethyl 1-(propylthio) propyl disulfide	M+2H	106.035807	210.057063	0.0004784	Organic disulphides
HMDB33049	Butyl 1-(methylthio)propyl disulfide	M+2H	106.035807	210.057063	0.0004784	Organic disulphides
HMDB15369	Chlorprothixene	M+3H	106.035559	315.084848	0.0007264	Benzothiopyrans
HMDB12488	1,2,3,4-Tetrahydro-beta-carboline	M+H+K	106.035241	172.100048	0.0010444	Indoles and derivatives
HMDB03929	5-Aminoimidazole	M+Na	106.037565	83.048347	0.0012796	Azoles
HMDB298	Cyromazine	M+2Na	106.037565	166.096694	0.0012796	Triazines

62						
HMDB40578	4-Thiocyanatophenol	M+2Na+H	106.038024	151.009184	0.0017386	Benzene and substituted derivatives
HMDB34413	1,2-Benzisothiazol-3(2H)-one	M+2Na+H	106.038024	151.009184	0.0017386	Benzothiazoles
HMDB06029	N-Acetylglutamine	M+H+Na	106.0381	188.079707	0.0018146	Carboxylic acids and derivatives

Figure 4.76: Boxplot Urinary Variable ID 12 Pre Surgery v Healthy Controls UHPLC-FTMS



p=8.53E-07

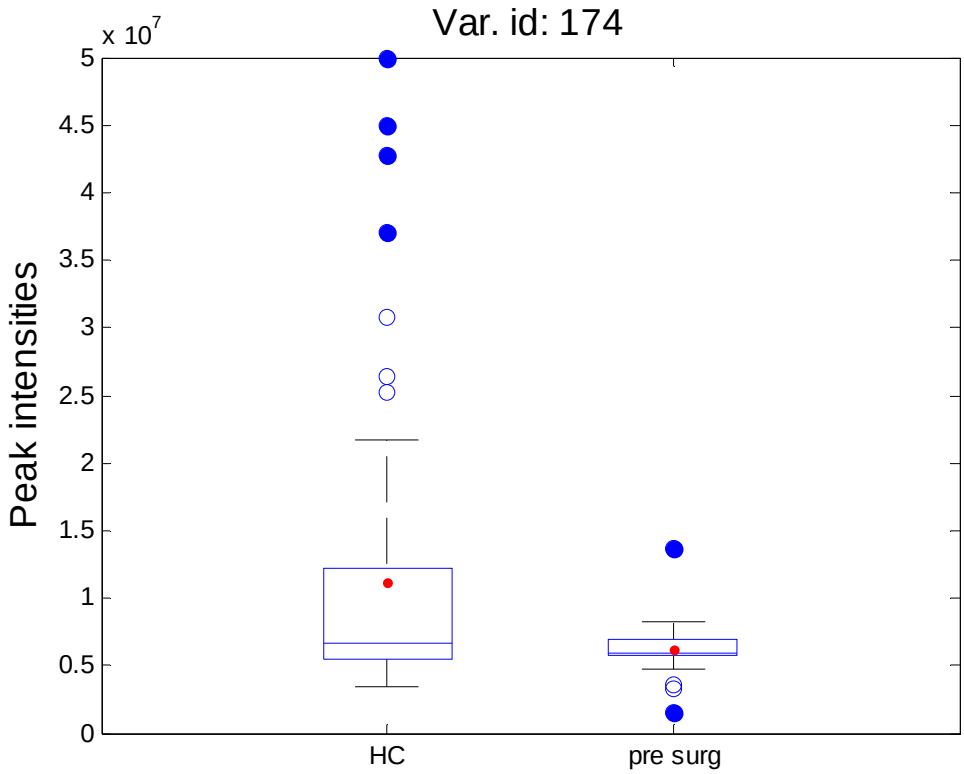
In this experiment, as previously discussed in Experiment 6.1 results, urinary peak intensities of variable ID 12 are higher in HCs than IBD patients, in this case those pre surgery.

Table 4.48.2: Mass spectra search for 153.0727774 m/z

Compound	**Name**	**Adduct**	**Adduct MW (Da)**	**Compound MW (Da)**	**Delta**	**Class**
HMDB37494	3'-Deoxyoleacein	M+2H	153.072813	304.131074	0.0000356	Benzene and substituted derivatives

HMDB29305	p-HPEA-EDA	M+2H	153.072813	304.131074	0.0000356	Benzene and substituted derivatives
HMDB35790	Matricarin	M+2H	153.072813	304.131074	0.0000356	Prenol lipids
HMDB14843	Delavirdine	M+3H	153.072062	456.194359	0.0007154	Diazinanes
HMDB34941	Artabsinolide D	M+H+Na	153.071609	282.146724	0.0011684	Prenol lipids
HMDB35846	Anguidol	M+H+Na	153.071609	282.146724	0.0011684	Prenol lipids
HMDB41324	Bisbynin	M+H+Na	153.071609	282.146724	0.0011684	Not classified
HMDB38660	Dihydrophaseic acid	M+H+Na	153.071609	282.146724	0.0011684	Prenol lipids
HMDB34983	Cynaratriol	M+H+Na	153.071609	282.146724	0.0011684	Prenol lipids
HMDB38661	Epidihydrophaseic acid	M+H+Na	153.071609	282.146724	0.0011684	Prenol lipids

Figure 4.77: Boxplot Urinary Variable ID 174 Pre Surgery v Healthy Controls UHPLC-FTMS



p=0.00026143

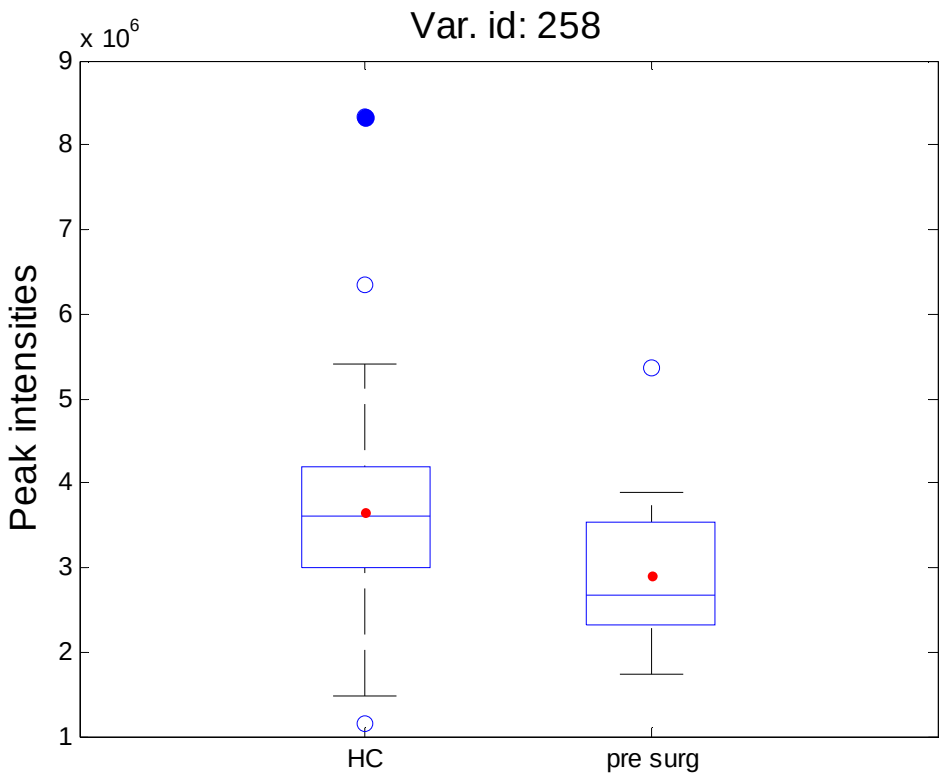
In this experiment, peak intensities of urinary variable 174 are higher in healthy controls than in pre surgery IBD patients. None of the metabolites identified are of biological relevance, however, most

are dietary and therefore the increased levels in HCs may be related to greater dietary variablitity in healthy controls when compared to IBD patients, as previously discussed.

Table 4.48.3: Mass spectra search for 169.1213976 m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB04705	12,13-DHOME	M+H+Na	169.121102	314.24571	0.0002956	Fatty Acyls
HMDB04704	9,10-DHOME	M+H+Na	169.121102	314.24571	0.0002956	Fatty Acyls
HMDB41220	Dibutyl decanedioate	M+H+Na	169.121102	314.24571	0.0002956	Fatty Acyls
HMDB31679	(9xi,10xi,12xi)-9,10-Dihydroxy-12-octadecenoic acid	M+H+Na	169.121102	314.24571	0.0002956	Fatty Acyls
HMDB00782	Octadecanedioic acid	M+H+Na	169.121102	314.24571	0.0002956	Fatty Acyls
HMDB32219	(+/-)-Dihydromintlactone	M+H	169.122306	168.11503	0.0009084	Not classified
HMDB05087	6-trans-Leukotriene B4	M+2H	169.122306	336.23006	0.0009084	Fatty Acyls
HMDB34670	6-Hydroxy-2,6-dimethyl-2,7-octadien-4-one	M+H	169.122306	168.11503	0.0009084	Prenol lipids
HMDB01184	Methyl propenyl ketone	2M+H	169.122306	84.057515	0.0009084	Carbonyl compounds
HMDB31607	1-Penten-3-one	2M+H	169.122306	84.057515	0.0009084	Carbonyl compounds

Figure 4.78: Boxplot Urinary Variable ID 258 Pre Surgery v Healthy Controls UHPLC-FTMS



p=2.81E-05

In this experiment, a higher peak intensity in urinary variable ID 258 is seen in healthy controls when compared to pre surgical IBD patients. 12,13-DHOME and 9,10-DHOME are identified as potentially biologically relevant. The other metabolites in this search are predominantly dietary.

12,13-DHOME and 9,10-DHOME

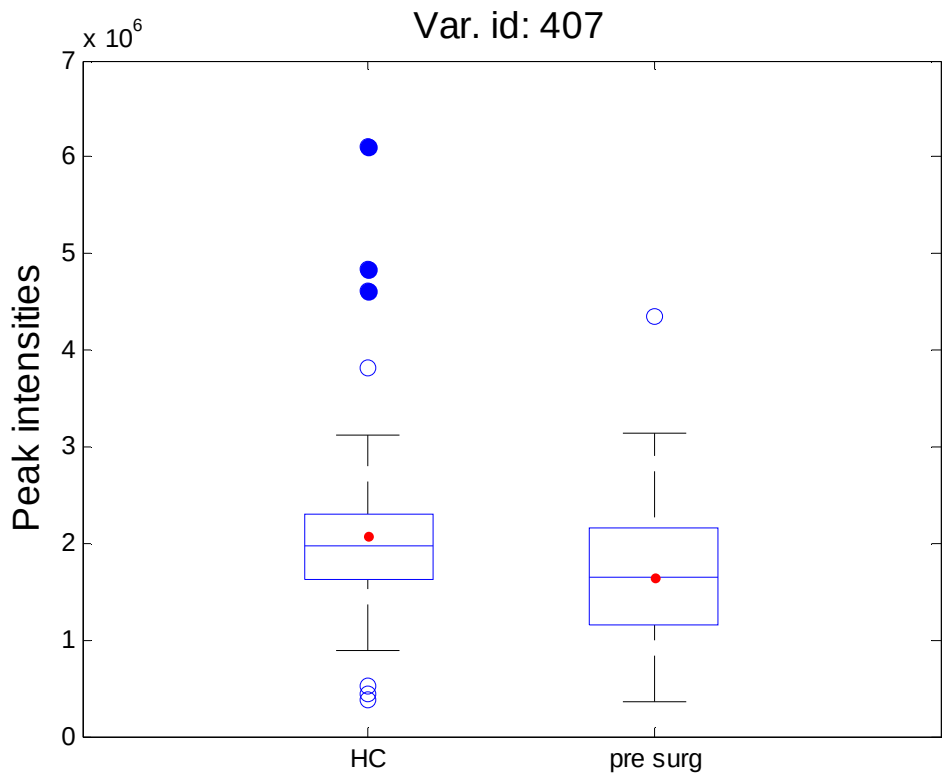
12,13-DHOME (dihydroxy-octadecenoic acid) and 9,10-DHOME are derivatives of linoleic acid diol that have been reported to be toxic in human tissue preparations. They are epoxide hydrolase metabolites of the leukotoxin12,13-EpOME (epoxy-octadecenoic acid) and 9,10-EpOME respectively. They belong to the class of organic compounds known as hydroxy fatty acids. Linoleic acid metabolites in this pathway are regarded as biologically active compounds. Leukotoxin (9(10)-epoxy-12Z-octadecenoic acid, 9,10 EpOME) and its regioisomer have been associated with multiple organ failure and adult respiratory distress syndrome seen in severe burn patients while the corresponding diols (9,10-DHOME and 12,13-DHOME) are also known to be cytotoxic. In healthy individuals, these metabolites may be endogenous chemical mediators regulating vascular permeability and inflammation (Yang, Schmelzer et al. 2009) In our study we see higher urinary levels in healthy controls than in pre-surgery IBD patients. This may represent increased excretion in

healthy controls. No direct links have been previously identified with IBD as yet but further investigation may be warranted.

Table 4.48.4: Mass spectra search for 200.1999862 m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB28964	Lysyl-Valine	M+2Na+H	200.202782	245.173942	0.0027958	Carboxylic acids and derivatives
HMDB29132	Valyl-Lysine	M+2Na+H	200.202782	245.173942	0.0027958	Not classified
HMDB40909	2-Hydroxy-22-methyltetracosanoic acid	M+2H	200.195274	398.375995	0.0047122	Fatty acyls
HMDB11529	LysoPE(24:6(6Z,9Z,12Z,15Z,18Z,21Z)/0:0)	M+H+2Na	200.205137	553.316839	0.0051508	Glycerophospholipids
HMDB11499	LysoPE(0:0/24:6(6Z,9Z,12Z,15Z,18Z,21Z))	M+H+2Na	200.205137	553.316839	0.0051508	Glycerophospholipids
HMDB15340	Dezocine	M+2Na+H	200.206804	245.177964	0.0068178	Tetralins
HMDB33599	Vignatic acid A	M+H+2Na	200.192457	553.278801	0.0075292	Carboxylic acids and derivatives
HMDB00378	2-Methylbutyroylcarnitine	M+2Na+H	200.191548	245.162708	0.0084382	Fatty acyls
HMDB41993	Pivaloylcarnitine	M+2Na+H	200.191548	245.162708	0.0084382	Fatty acyls
HMDB13128	Valerylcarnitine	M+2Na+H	200.191548	245.162708	0.0084382	Fatty acyls

Figure 4.79: Boxplot Urinary Variable ID 407 Pre Surgery v Healthy Controls UHPLC-FTMS



p=0.00067961

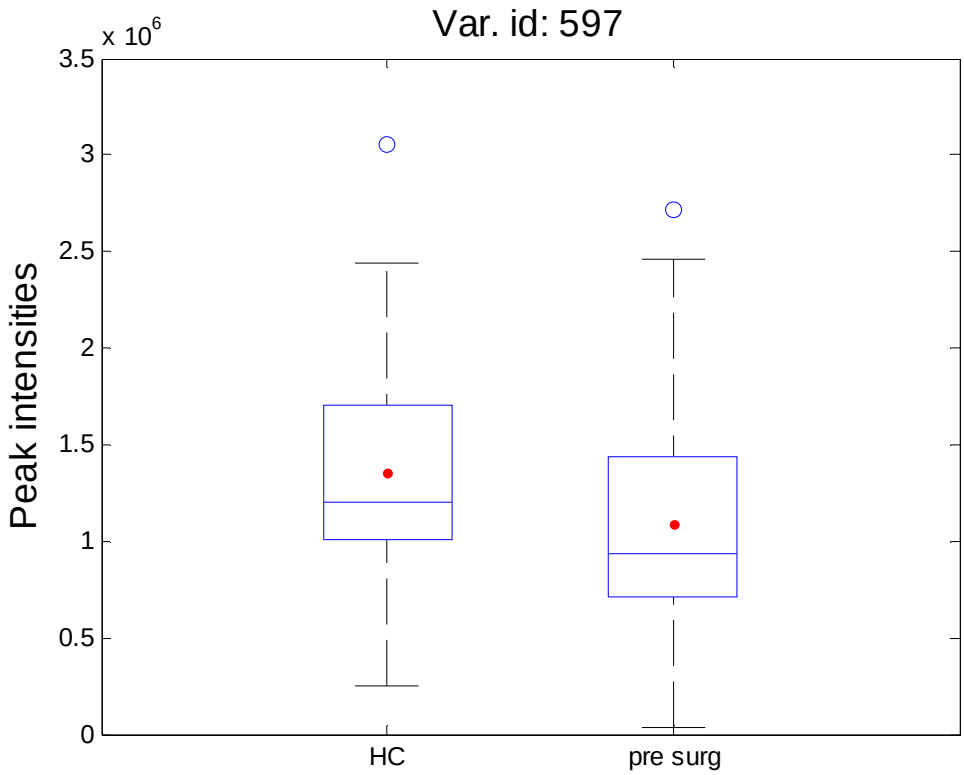
In this experiment, a higher peak intensity in urinary variable ID 407 is seen in healthy controls when compared to pre surgical IBD patients. None of the metabolites identified have biological links to IBD. The difference identified may be dietary.

Table 4.48.5: Mass spectra search for 253.0654594 m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB29968	Ethyl beta-D-glucopyranoside	M+2Na-H	253.065848	208.094688	0.0003886	Carbohydrates and carbohydrate conjugates
HMDB33942	Dambonitol	M+2Na-H	253.065848	208.094688	0.0003886	Alcohols and polyols
HMDB15438	Spirapril	M+H+K	253.065024	466.159613	0.0004354	Carboxylic acids and derivatives
HMDB13969	Zileuton sulfoxide	M+H	253.064139	252.056863	0.0013204	Not classified
HMDB37373	Quercetin 3-(3",6"-di-p-coumarylgl	M+3H	253.063621	756.169035	0.0018384	Not classified

	ucoside)					
HMDB28970	Methionyl-Cysteine	M+H	253.06751	252.060234	0.0020506	Carboxylic acids and derivatives
HMDB28781	Cysteinyl-Methionine	M+H	253.06751	252.060234	0.0020506	Carboxylic acids and derivatives
HMDB14733	Oxytetracycline	M+2Na	253.063308	460.14818	0.0021514	Tetracyclines
HMDB33138	Methyl salicylate O-[rhamnosyl-(1->6)-glucoside]	M+2Na	253.068256	460.158076	0.0027966	Carbohydrates and carbohydrate conjugates
HMDB29172	3-keto-2-Methylbutyrate	2M+Na	253.068256	115.039519	0.0027966	Not classified

Figure 4.80: Boxplot Urinary Variable ID 597 Pre Surgery v Healthy Controls UHPLC-FTMS



p=0.00079279

In this experiment, a higher peak intensity in urinary variable ID 597 is seen in healthy controls when compared to pre surgical IBD patients. Methionyl-Cysteine, Cysteinyl-Methionine and Oxytetracycline may be of biological relvance. The other metabolites idenitifed represent dietary intake.

Methionyl-Cysteine and Cysteinyl-Methionine

Methionyl-Cysteine and Cysteinyl-Methionine are dipeptides composed of methionine and cysteine. They are incomplete breakdown products of protein digestion or protein catabolism. Metabolism of the sulfur-containing amino acid cysteine is increased in both UC and CD, and metabolism of cysteine and methionine is increased in ileal CD. This is accompanied by increases in riboflavin metabolism, glutathione transporters, and the N-acetylgalactosamine phosphotransferase system. Mucin, which is rich in cysteine and glycosylated sugars, is abundant in the intestinal epithelium, and it is upregulated during inflammation. The increases in cysteine metabolism and N-acetylgalactosamine transporters may reflect a shift in the microbiome towards greater abundance of microbes that use mucin as a primary energy source. This functionality suggests activity at the mucosa and this may be problematic for a damaged IBD epithelium with compromised barrier function (Morgan, Tickle et al. 2012). In our study we see less Methionyl-Cysteine and Cysteinyl-Methionine in the urine of IBD patients. This may reflect the upregulated metabolism resulting in less excretion.

Oxytetracycline

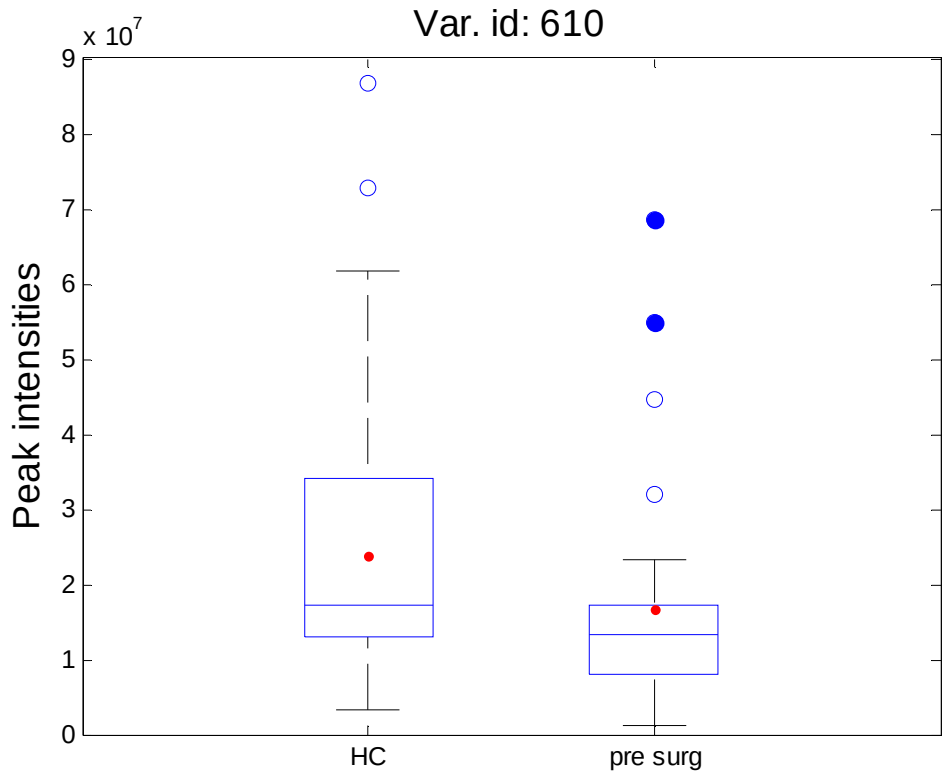
Oxytetracycline, a broad-spectrum antibiotic, belongs to the class of organic compounds known as tetracyclines. Some evidence suggests that there may be a link between oral tetracycline usage and the development of IBD, particularly CD (Margolis, Fanelli et al. 2010). This metabolite would only be identified from the urine of those taking this drug during the study therefore we cannot derive any causal effect from this data.

Table 4.48.6: Mass spectra search for 257.1488092 m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB38078	1,1-Diethoxy-2,6-nonadiene	M+2Na-H	257.14879	212.17763	0.0000192	Ethers
HMDB37626	1-Ethylhexyl tiglate	M+2Na-H	257.14879	212.17763	0.0000192	Fatty acyls
HMDB34286	Ethyl 10-undecenoate	M+2Na-H	257.14879	212.17763	0.0000192	Fatty acyls
HMDB37226	Citronellyl propionate	M+2Na-H	257.14879	212.17763	0.0000192	Fatty acyls
HMDB40451	Oxacyclotetradecan-2-one	M+2Na-H	257.14879	212.17763	0.0000192	Macrolides and analogues
HMDB32792	(±)-(E)-3-Methyl-4-decen-1-yl acetate	M+2Na-H	257.14879	212.17763	0.0000192	Fatty acyls
HMDB31028	Methyl (E)-2-dodecenoate	M+2Na-H	257.14879	212.17763	0.0000192	Fatty acyls

HMDB37187	Rhodinyl propionate	M+2Na-H	257.14879	212.17763	0.0000192	Fatty acyls
HMDB39805	4-(3-Hydroxybutyl)-3,3,5-trimethylcyclohexanone	M+2Na-H	257.14879	212.17763	0.0000192	Prenol lipids
HMDB31741	Coniferan	M+2Na-H	257.14879	212.17763	0.0000192	Not classified

Figure 4.81: Boxplot Urinary Variable ID 610 Pre Surgery v Healthy Controls UHPLC-FTMS



p=6.65E-05

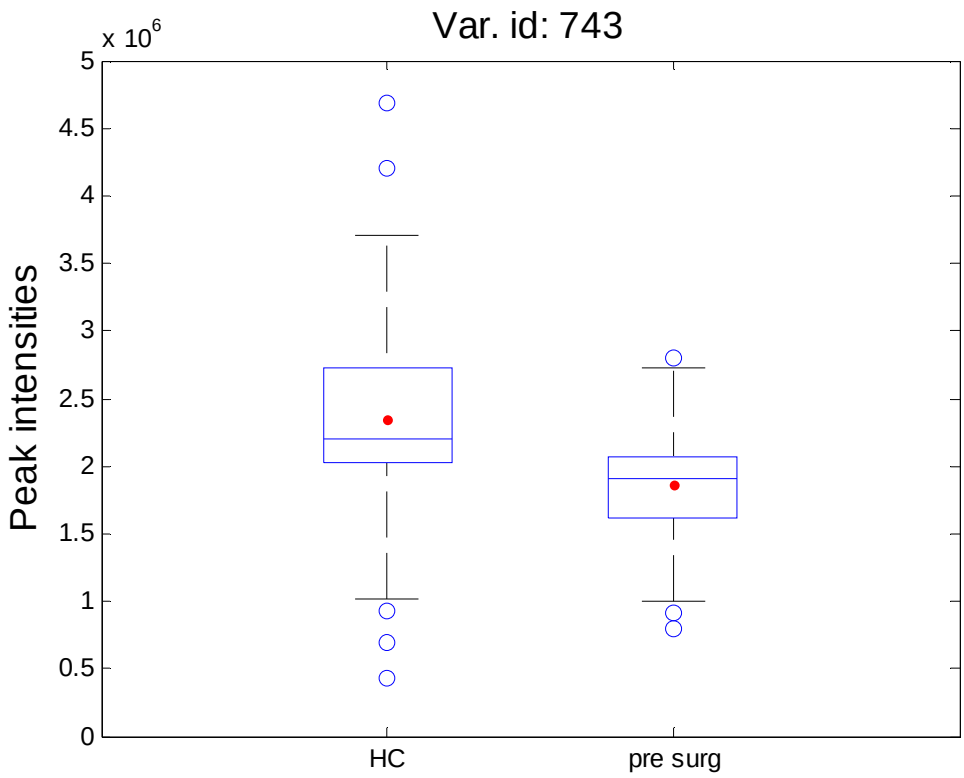
In this experiment, a higher peak intensity in urinary variable ID 610 is seen in healthy controls when compared to pre surgical IBD patients. None of the urinary metabolites identified are relevant in IBD, and the differences between the groups are likely to be dietary in nature.

Table 4.48.7: Mass spectra search for 302.1587031 m/z

Compound	**Name**	**Adduct**	**Adduct MW (Da)**	**Compound MW (Da)**	**Delta**	**Class**
HMDB41889	Etamiphylline	M+Na	302.158743	279.169525	0.0000399	Not available (Super Class Alkaloids

						and derivatives)
HMDB15385	Lisdexamfetamine	M+K	302.16292	263.199762	0.0042169	Carboxylic acids and derivatives
HMDB30459	Hordatine B	M+H+Na	302.15433	580.312166	0.0043731	2-arylbenzofuran flavonoids
HMDB15237	Sibutramine	M+Na	302.164596	279.175378	0.0058929	Benzene and substituted derivatives
HMDB14339	Tramadol	M+K	302.151687	263.188529	0.0070161	Benzene and substituted derivatives
HMDB15646	Desvenlafaxine	M+K	302.151687	263.188529	0.0070161	Not classified
HMDB60532	O-Desmethylvenlafaxine	M+K	302.151687	263.188529	0.0070161	Not classified
HMDB29567	Hydroxy-alpha-sanshool	M+K	302.151687	263.188529	0.0070161	Alcohols and polyols
HMDB13892	N-Desmethylvenlafaxine	M+K	302.151687	263.188529	0.0070161	Not classified
HMDB15273	Doxepin	M+Na	302.151532	279.162314	0.0071711	Benzoxepines

Figure 4.82: Boxplot Urinary Variable ID 743 Pre Surgery v Healthy Controls UHPLC-FTMS



p=0.00040501

In this experiment, a higher peak intensity in urinary variable ID 743 is seen in healthy controls when compared to pre surgical IBD patients. None of the urinary metabolites identified are relevant in IBD as previously discussed in experiment 9.1.

Table 4.48.8: Mass spectra search for 322.075723 m/z

Compound	**Name**	**Adduct**	**Adduct MW (Da)**	**Compound MW (Da)**	**Delta**	**Class**
HMDB02044	8-Hydroxyguanosine	M+Na	322.075801	299.086583	0.000078	Purine nucleosides
HMDB41818	7-Aminoflunitrazepam	M+K	322.075248	283.11209	0.000475	Benzodiazepines
HMDB13220	Beta-Citryl-L-glutamic acid	M+H	322.076872	321.069596	0.001149	Carboxylic acids and derivatives
HMDB12947	Ferrocytochrome	M+H+K	322.073866	604.177298	0.001857	Not classified
HMDB29177	3'-O-Methyl-(-)-epicatechin-7-O-sulphate	M+2Na+H	322.077603	367.048763	0.00188	Flavanoids

HMDB61336	Teniposide catechol derivative	M+2H	322.077632	642.140712	0.001909	Lignan lactones
HMDB61280	Tenofovir Monophosphate	M+2Na+H	322.073511	367.044671	0.002212	Imidazopyimidines
HMDB37850	Myricetin 3,3'-digalactoside	M+2H	322.078883	642.143214	0.00316	Not classified
HMDB60815	Desmethyl frovatriptan	M+2K+H	322.071852	245.152812	0.003871	Indoles and derivatives
HMDB14618	Chlordiazepoxide	M+Na	322.071758	299.08254	0.003965	Benzodiazepines

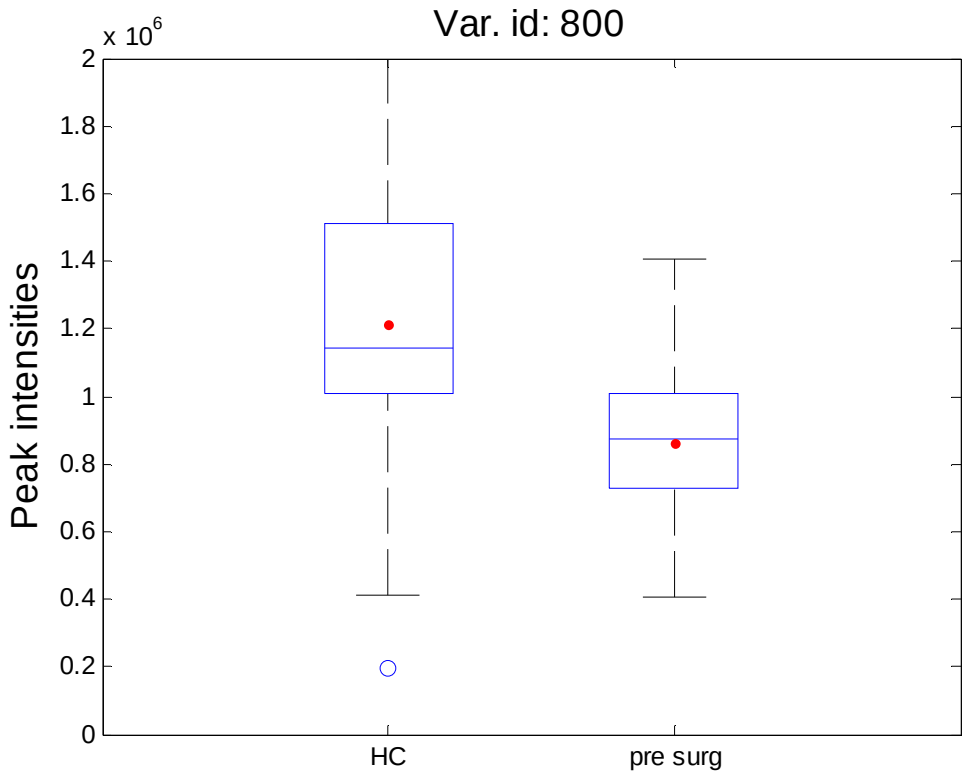
As previously discussed in experiment 9.1 none of the urinary metabolites identified are of biological relevance in IBD but 8-hydroxyguanosine and Myricetin 3,3'-digalactoside may warrent further investigation as potential biomarkers.

Table 4.48.9: Mass spectra search for 334.0909909 m/z

Compound	**Name**	**Adduct**	**Adduct MW (Da)**	**Compound MW (Da)**	**Delta**	**Class**
HMDB30704	Taxiphyllin	M+Na	334.08972	311.100502	0.0012709	Carbohydrates and carbohydrate conjugates
HMDB60471	Dhurrin	M+Na	334.08972	311.100502	0.0012709	Carbohydrates and carbohydrate conjugates
HMDB37841	N-(1-Deoxy-1-fructosyl)methionine	M+Na	334.093091	311.103873	0.0021001	Carbohydrates and carbohydrate conjugates
HMDB15585	Chlophedianol	M+2Na-H	334.094502	289.123342	0.0035111	Benzene and substituted derivatives
HMDB60463	Citalopram propionic acid	M+Na	334.08499	311.095772	0.0060009	Not classified
HMDB14045	3-Methoxymorphinan	M+2K+H	334.097004	257.177964	0.0060131	Morphinans
HMDB60552	Dextrorphan	M+2K+H	334.097004	257.177964	0.0060131	Morphinans
HMDB14992	Levorphanol	M+2K+H	334.097004	257.177964	0.0060131	Morphinans
HMDB39087	Sudachiin B	M+2H	334.097076	666.1796	0.0060851	Flavonoids
HMDB390	Sudachiin C	M+2H	334.097076	666.1796	0.0060851	Not

88						classified

Figure 4.83: Boxplot Urinary Variable ID 800 Pre Surgery v Healthy Controls UHPLC-FTMS



p=1.02E-06

In this experiment, higher peak intensities of urinary variable ID 800 are in seen in healthy controls than in pre-surgery IBD patients. This was identified and discussed previously in experiment 6.1, and is likely related to diet.

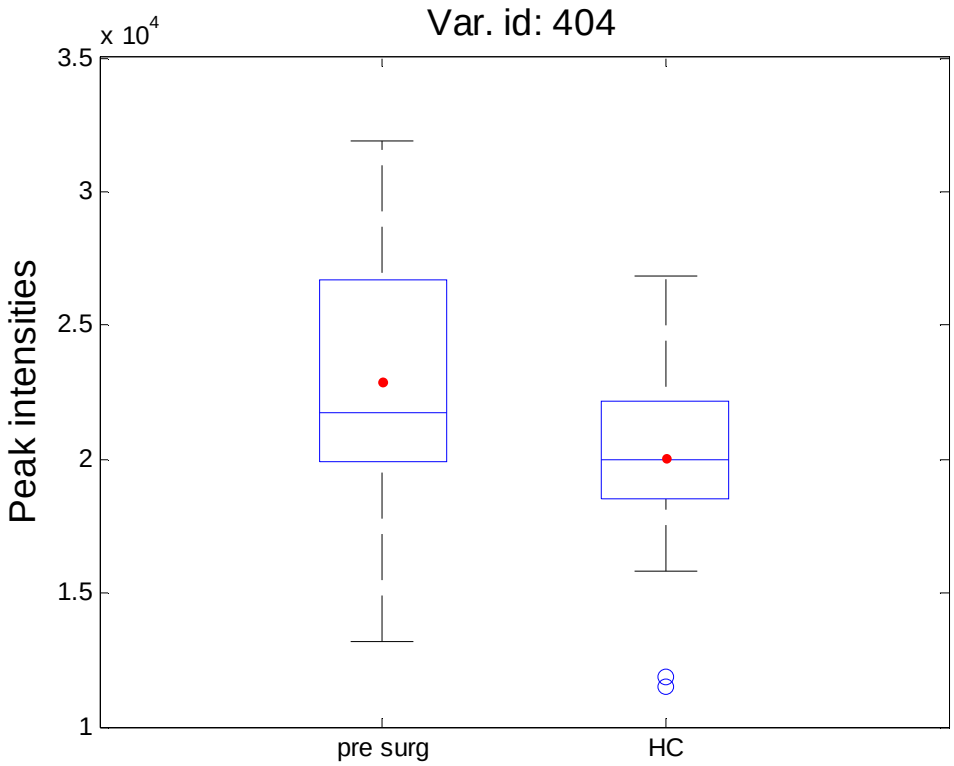
4.15.2 Experiment 13.2: Metabolite Identification Pre-Surgery v Healthy Controls GC-ToF-MS

Table 4.49: Putative Metabolites Pre Surgery v Healthy Controls GC-ToF-MS

Variable ID	Biofluid	Quant mass	Retention time	Putative match	p value	q value
8	Serum	47	573.478	Unknown	7.03E-08	1.91E-05
14	Serum	49	577.428	Unknown	4.48E-05	0.0121856
22	Serum	51	575.128	Unknown	3.88E-05	0.0105536
27	Serum	52	571.628	Threonine or urea	1.74E-09	4.73E-07
30	Serum	53	575.774	Unknown	4.25E-08	1.16E-05
34	Serum	54	577.453	Unknown	8.69E-09	2.36E-06
45	Serum	56	574.878	Unknown	1.18E-08	3.21E-06
60	Serum	60	570.318	Urea	8.74E-10	2.38E-07
64	Serum	61	575.528	Unknown	4.60E-08	1.25E-05
69	Serum	63	576.428	Unknown	8.14E-07	0.000221408
74	Serum	64	570.778	Urea	3.15E-09	8.57E-07
76	Serum	65	571.903	Threonine or urea	1.12E-09	3.05E-07
81	Serum	66	571.278	Threonine or urea	4.55E-10	1.24E-07
86	Serum	67	572.201	Unknown	3.81E-09	1.04E-06
93	Serum	69	571.028	Threonine or urea	2.52E-09	6.85E-07
98	Serum	70	561.694	Dihydroxybutanoic acid or Serine	9.56E-09	2.60E-06
106	Serum	71	571.978	Threonine or urea	1.22E-09	3.32E-07
107	Serum	72	572.378	Unknown	2.19E-09	5.96E-07
121	Serum	75	574.929	Unknown	6.90E-07	0.00018768
133	Serum	78	572.928	Unknown	9.13E-09	2.48E-06
137	Serum	79	570.426	Urea	3.19E-08	8.68E-06
142	Serum	80	570.674	Urea	2.07E-09	5.63E-07
157	Serum	84	575.428	Unknown	6.23E-08	1.69E-05
218	Serum	99	570.728	Urea	4.52E-10	1.23E-07
221	Serum	100	562.088	Dihydroxybutanoic acid or Serine	8.26E-09	2.25E-06
228	Serum	102	563.626	Dihydroxybutanoic acid or Serine	6.42E-07	0.000174624
266	Serum	111	571.579	Threonine or urea	7.15E-09	1.94E-06
279	Serum	115	572.628	Unknown	1.04E-08	2.83E-06
334	Serum	127	571.479	Threonine or urea	5.89E-09	1.60E-06
355	Serum	132	576.428	Unknown	1.37E-08	3.73E-06
383	Serum	139	570.378	Urea	0.00015774	0.04290528
390	Serum	141	570.928	Urea	3.15E-09	8.57E-07
394	Serum	141	793.553	Galactose	4.14E-05	0.0112608
404	Serum	144	532.088	Octanoic acid	2.25E-06	0.000612
446	Serum	155	570.524	Urea	3.66E-09	9.96E-07

455	Serum	157	570.478	Urea	3.25E-10	8.84E-08
507	Serum	171	570.474	Urea	6.33E-10	1.72E-07
509	Serum	172	570.678	Urea	9.12E-10	2.48E-07
513	Serum	173	570.328	Urea	9.02E-11	2.45E-08
516	Serum	174	568.138	Urea	2.10E-09	5.71E-07
549	Serum	183	795.628	Citric acid	5.93E-06	0.00161296
554	Serum	184	792.678	Galactose	4.80E-05	0.013056
563	Serum	186	570.728	Urea	1.35E-08	3.67E-06
569	Serum	187	571.628	Threonine or urea	7.15E-08	1.94E-05
574	Serum	189	570.078	Urea	5.09E-11	1.38E-08
576	Serum	190	569.928	Urea	8.01E-11	2.18E-08
582	Serum	191	569.826	Urea	1.78E-08	4.84E-06
622	Serum	200	537.679	Unknown	3.51E-05	0.0095472
637	Serum	204	568.779	Urea	3.74E-08	1.02E-05
642	Serum	206	575.028	Unknown	3.38E-05	0.0091936
754	Serum	237	902.428	Linoleic acid	3.00E-05	0.00816
819	Serum	257	794.778	Galactose	1.20E-06	0.0003264
823	Serum	259	794.828	Galactose	1.40E-05	0.003808
831	Serum	261	570.453	Urea	4.50E-06	0.001224
844	Serum	265	845.578	Fructose	2.77E-06	0.00075344
864	Serum	272	798.856	Unknown	0.00012309	0.03348048
867	Serum	273	795.729	Citric acid	3.36E-05	0.0091392
883	Serum	280	864.078	Tyrosine	0.00016737	0.04552464
990	Serum	318	829.026	Glucuronic acid	1.45E-06	0.0003944
1000	Serum	324	967.778	Oleic acid	0.00017291	0.04703152
1056	Serum	347	795.178	Pentanoic acid	9.34E-05	0.0254048
1059	Serum	349	794.328	Galactose	4.55E-05	0.012376
1072	Serum	353	954.878	Unknown	6.13E-05	0.0166736
1099	Serum	363	795.678	Citric acid	6.55E-05	0.017816
1113	Serum	369	957.078	Unknown	5.67E-05	0.0154224
1146	Serum	382	954.428	Unknown	8.94E-05	0.0243168
1149	Serum	383	956.728	Unknown	2.37E-05	0.0064464
1151	Serum	384	957.378	Unknown	1.00E-04	0.0272
1325	Serum	432	841.678	myo-inositol	0.00013905	0.0378216
1344	Serum	441	954.628	Unknown	0.00016304	0.04434688
1392	Serum	455	954.528	Unknown	0.00017072	0.04643584
1395	Serum	456	954.578	Unknown	0.0001677	0.0456144
82	Urine	77	915.078	Unknown	0.0017361	0.0486108
320	Urine	231	595.9715	Malonic acid or oxalic acid	1.66E-05	0.0004648
324	Urine	233	593.988	Erythritol	0.00010083	0.00282324

Figure 4.84: Boxplot Serum Variable ID 404 Octanoic Acid Pre Surgery v Healthy Controls GC-ToF-MS

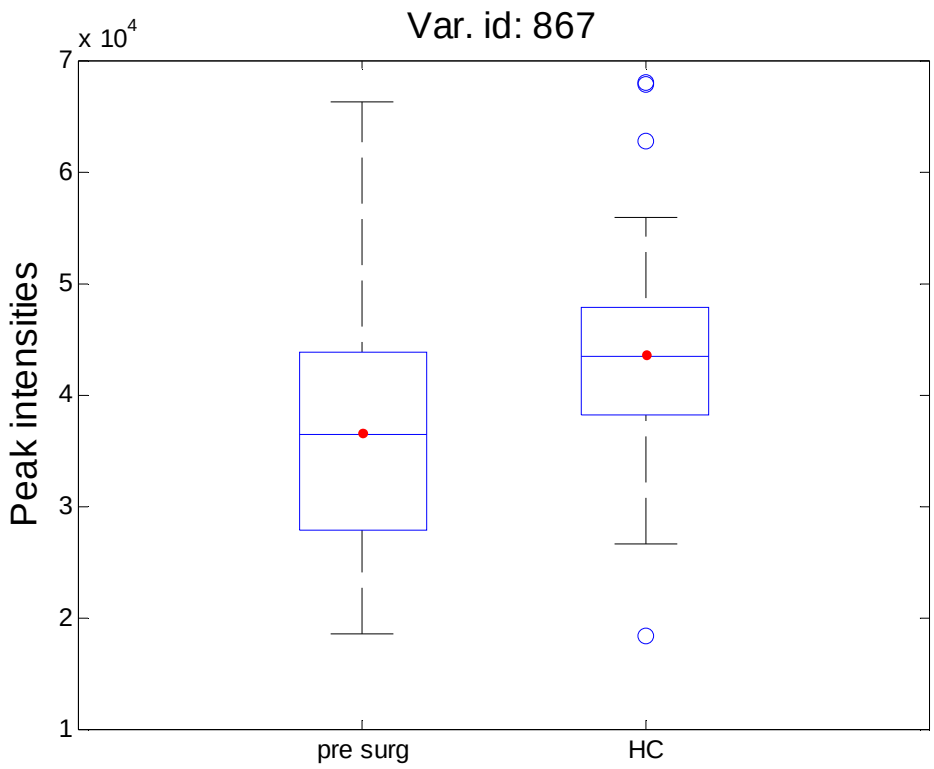


p=2.25E-06

Octanoic Acid

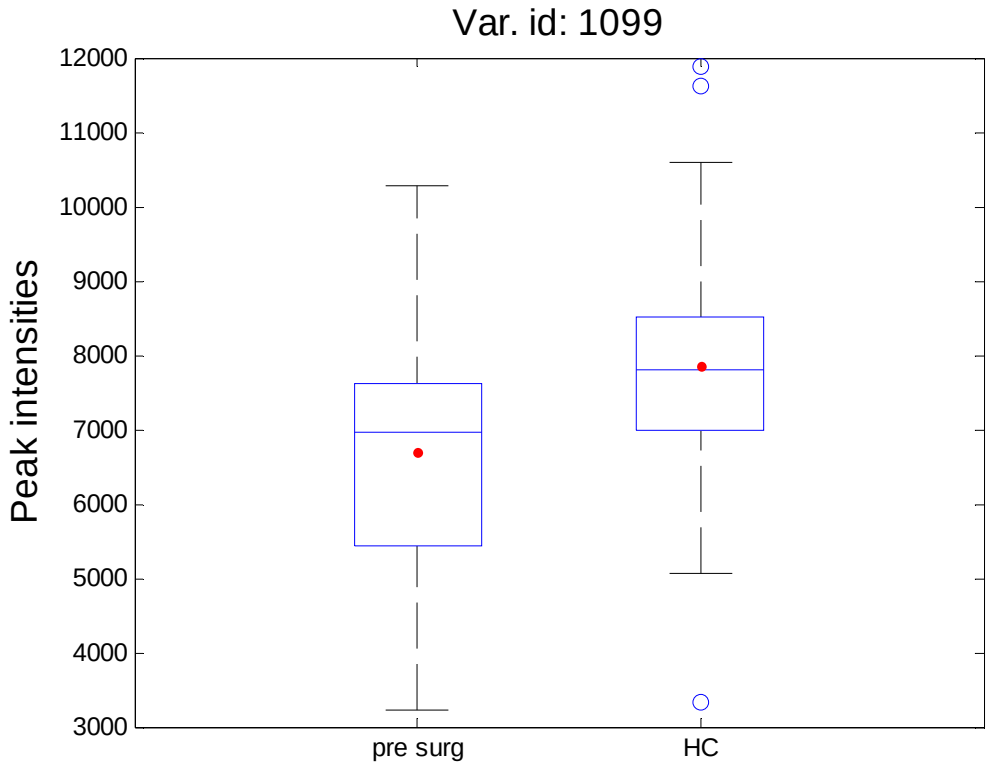
In this experiment, variable ID 404, identified as octanoic acid, is increased in the serum of pre surgery IBD patients compared to healthy controls. It belongs to the class of organic compounds known as fatty acid esters. It is a medium chain fatty acids and has been shown to be significantly decreased in patients with CD, UC and pouchitis compared with healthy controls (De Preter, Machiels et al. 2015). Our findings are not in keeping with this, showing increased levels in the serum of pre surgery IBD patients.

Figure 4.85: Boxplot Serum Variable ID 867 Citric Acid Pre Surgery v Healthy Controls GC-ToF-MS



p= 3.36E-05

Figure 4.86: Boxplot Serum Variable ID 1099 Citric Acid Pre Surgery v Healthy Controls GC-ToF-MS

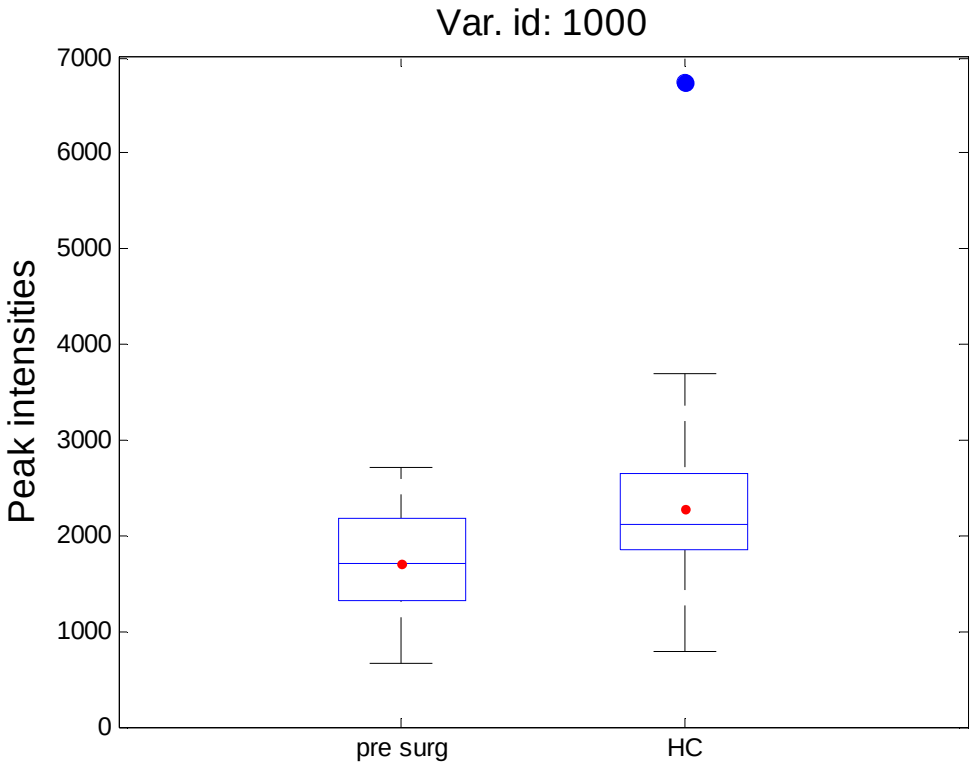


p=6.55E-05

Citric Acid

The variable IDs 867 and 1099, identified as citric acid, are reduced in the serum of pre surgery IBD patients compared to healthy controls. Citric acid is a weak acid that is an intermediate in the tricarboxylic acid cycle or that may be introduced with diet. Acute colitis has been shown to result in depletion in the levels of gut microbial cometabolites in urine along with an increase in citric acid cycle intermediates. These findings suggest that DSS-induced acute colitis causes a disturbance of lipid and energy metabolism, damage to the colon and liver, a promoted antioxidative and anti-inflammatory response, and perturbed gut microbiotal communities (Dong, Zhang et al. 2013). Our results show reduced levels of citric acid in the serum of pre surgery IBD patients. This may be due to the increased utilisation of citric acid in the tricarboxylic acid cycle during periods of inflammation.

Figure 4.87: Boxplot Serum Variable ID 1000 Oleic Acid Pre Surgery v Healthy Controls GC-ToF-MS

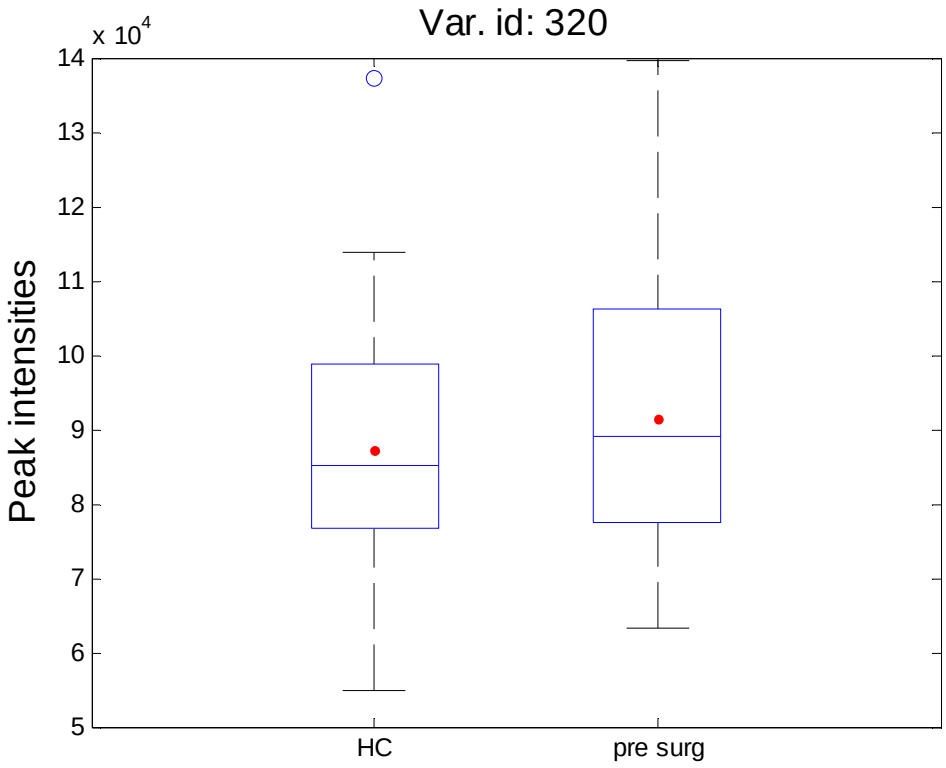


p=0.00017291

Oleic Acid

The variable ID 1000 identified as oleic acid, is reduced in the serum of pre surgery IBD patients compared to healthy controls. Oleic acid, a fatty acyl, is an unsaturated fatty acid that is the most widely distributed and abundant fatty acid in nature. It belongs to the class of organic compounds known as long-chain fatty acids, and has been shown to be decreased in the colonic mucosa of IBD patients (Ramakers, Mensink et al. 2007). Our results show reduced levels in the serum of pre surgery IBD patients compared to HCs, which is in keeping with these previous findings.

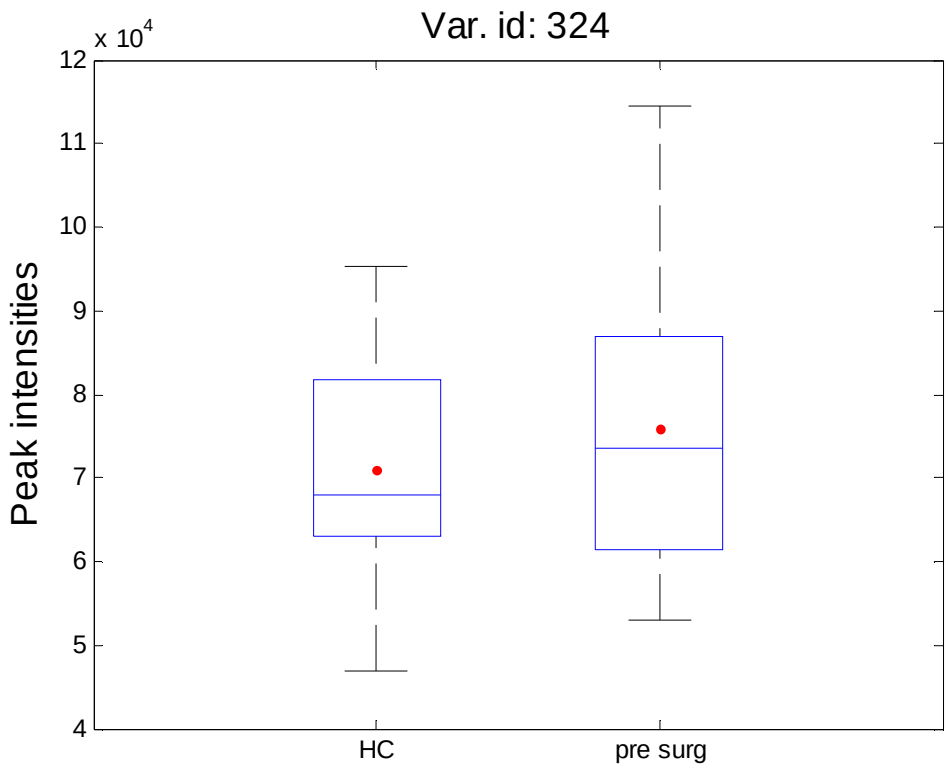
Figure 4.88: Boxplot Urinary Variable ID 320 Malonic acid or Oxalic acid Pre Surgery v Healthy Controls GC-ToF-MS



p=1.66E-05

Increased urinary levels of variable ID 320, Malonic Acid or Oxalic Acid, are seen in pre surgery IBD patients compared to HCs. Both compounds belong to the class of carboxylic acids and derivatives. Oxalic acid is found in plants and vegeteables and these finding may be dietary in nature. Malonic acid may be either a drug metabolite or endogenous. Neither are of biological relevance in IBD.

Figure 4.89: Urinary Variable ID 324 Erythritol Pre Surgery v Healthy Controls GC-ToF-MS



p=0.00010083

As previously discussed, urinary erythritol levels seen here to be higher in pre surgery IBD patients than HCs, are dietary in nature and likely not of relevance in IBD.

4.15.3 Experiment 13 Summary

Metabolites in the classes organic disulphides, benzene and substituted benzene derivatives, carboxylic acids and derivatives, azoles, and fatty acyls have been shown to be decreased in both urine and serum samples of pre surgery IBD patients in comparison to healthy controls. Metabolites in the class carbohydrates and carbohydrate conjugates are increased in the urine of pre surgery IBD patients compared to healthy controls.

4.16 Experiment 14: Metabolite Identification: Post Surgery v Healthy Controls

In this experiment we aim to determine whether we can identify metabolites that differentiate between post surgery IBD patients and HCs.

Table 4.50 Experiment 14 number of samples analysed

	Post-surgery	**Healthy Controls**
Serum samples	28	62
Urine samples	28	60

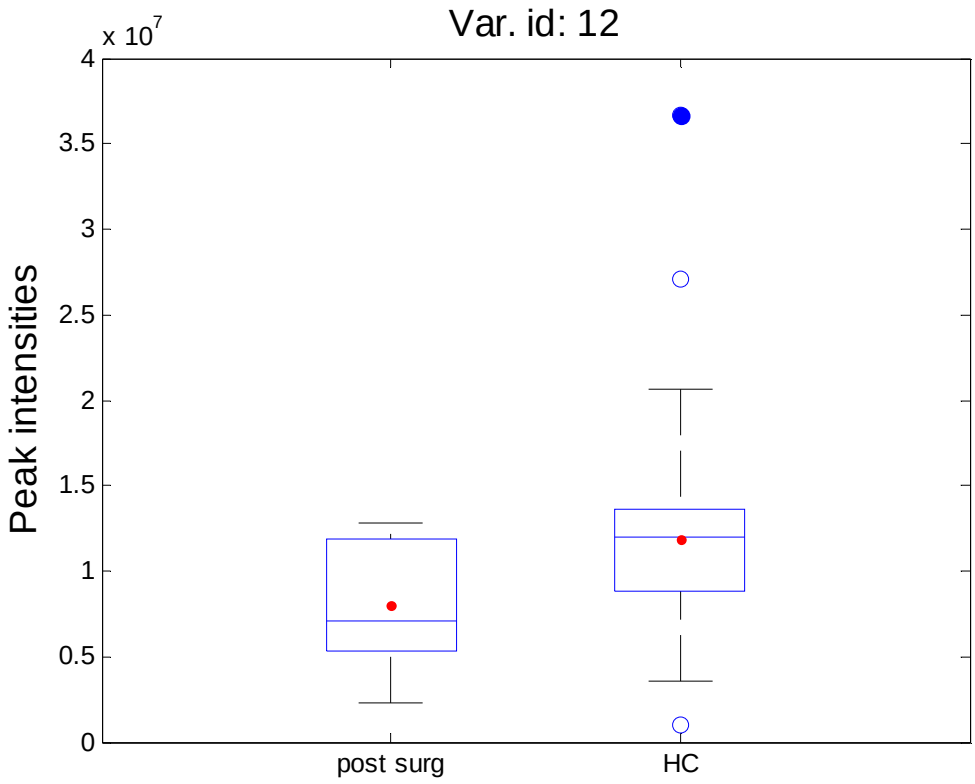
4.16.1 Experiment 14.1 Metabolite Identification: Post Surgery v Healthy Controls UHPLC-FTMS

Table 4.51: Important Variables Identified Post Surgery v Healthy Controls UHPLC-FTMS

Variable ID	**Biofluid**	**M/z**	**Retention time**	**p value**	**q value**
12	Urine	106.0362854	240.0221	0.00024696	0.0111132
125	Urine	143.1058707	371.7795	0.00030249	0.01361205
450	Urine	209.1244076	168.6093	2.66E-05	0.001197
544	Urine	237.1222813	180.762	0.00029484	0.0132678
548	Urine	239.088045	336.9686	0.00063886	0.0287487
751	Urine	303.2348021	502.1351	0.00040589	0.01826505
800	Urine	334.0909909	299.601	0.00012066	0.0054297

The variables that have been previously identified and discussed (variable ID 12 and 800) are not repeated, although the relevant boxplots are displayed to show the difference between the groups (post surgery IBD patients and healthy controls) in this experiment.

Figure 4.90: Boxplot Urinary Variable ID 12 Post Surgery v Healthy Controls UHPLC- FTMS



p=0.00024696

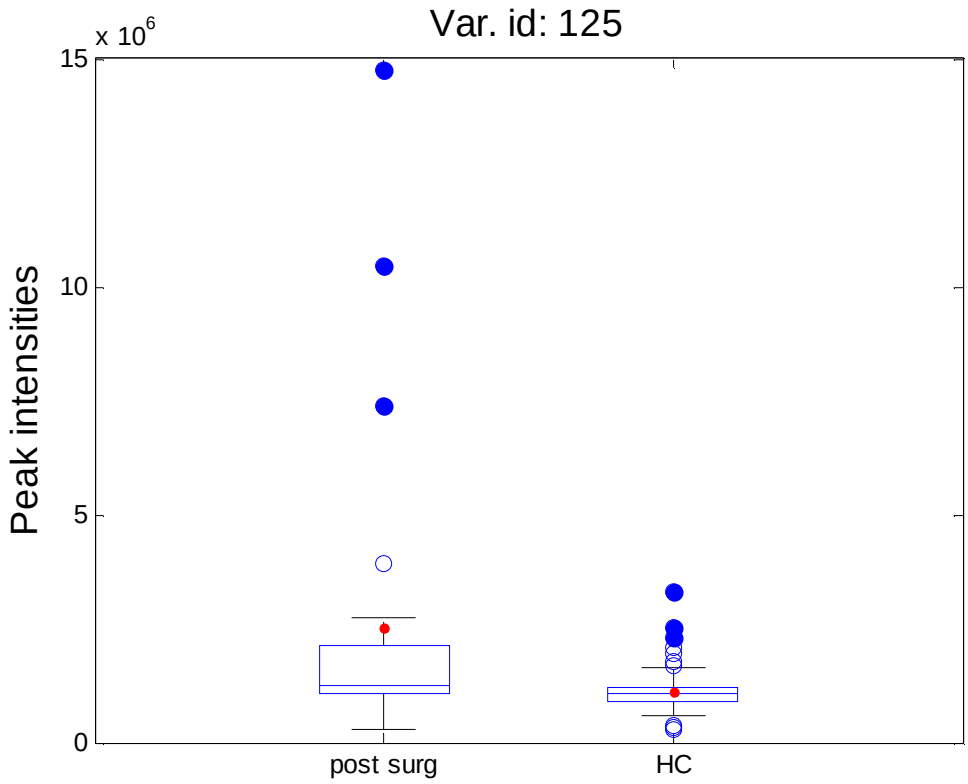
In this study urinary variable ID 12 is seen to be reduced in post surgical IBD patients compared to healthy controls. These metabolites have been discussed previously.

Table 4.51.1: Mass spectra search for 143.1058707 m/z

Compound	**Name**	**Adduct**	**Adduct MW (Da)**	**Compound MW (Da)**	**Delta**	**Class**
HMDB40215	cis-3-Hexenyl acetate	M+H	143.106656	142.09938	0.0007853	Carboxylic acids and derivatives
HMDB01568	trans-2-Octenoic acid	M+H	143.106656	142.09938	0.0007853	Fatty acyls
HMDB31293	2,3-Octanedione	M+H	143.106656	142.09938	0.0007853	Carbonyl compounds
HMDB13904	(3E)-2-Propylpent-3-enoic acid	M+H	143.106656	142.09938	0.0007853	Not classified
HMDB32268	Ethyl 2E-hexenoate	M+H	143.106656	142.09938	0.0007853	Fatty acyls
HMDB37630	xi-Tetrahydro-3-propyl-2H-pyran-2-one	M+H	143.106656	142.09938	0.0007853	Lactones

HMDB31403	Cyclohexaneacetic acid	M+H	143.106656	142.09938	0.0007853	Carboxylic acids and derivatives
HMDB29761	Ethyl (±)-2-methyl-4-pentenoate	M+H	143.106656	142.09938	0.0007853	Fatty acyls
HMDB40212	2-Hexenyl acetate	M+H	143.106656	142.09938	0.0007853	Carboxylic acids and derivatives
HMDB00392	2-Octenoic acid	M+H	143.106656	142.09938	0.0007853	Fatty acyls

Figure 4.91: Boxplot Urinary Variable ID 125 Post Surgery v Healthy Controls UHPLC- FTMS



p=0.00030249

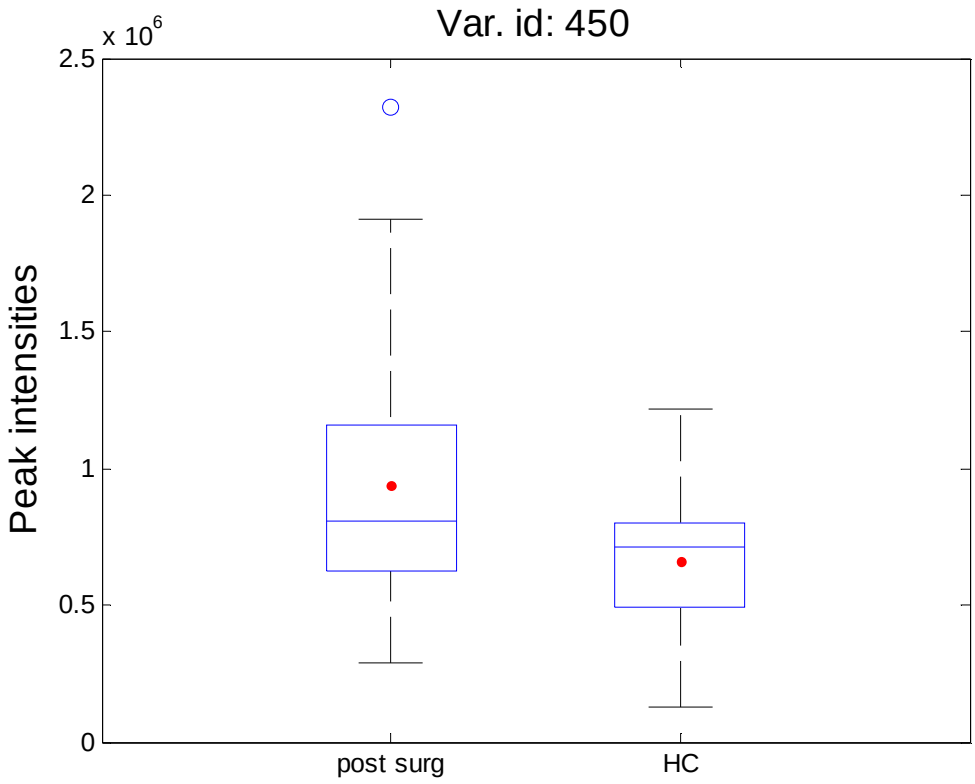
In this study urinary variable ID 125 is seen to be increased in post surgical IBD patients compared to healthy controls. All of the above metabolites are dietary in nature.

Table 4.51.2: Mass spectra search for 209.1244076m/z

Compound	**Name**	**Adduct**	**Adduct MW (Da)**	**Compound MW (Da)**	**Delta**	**Class**
HMDB02006	2,3-Diaminopropionic acid	2M+H	209.124431	104.058578	0.0000234	Carboxylic acids and derivatives
HMDB11579	MG(20:4(8Z,11Z,14Z,	M+H+K	209.123722	378.27701	0.0006856	Glycerolipids

	17Z)/0:0/0:0)					
HMDB04666	2-Arachidonylglycerol	M+H+K	209.123722	378.27701	0.0006856	Glycerolipids
HMDB11549	MG(0:0/20:4(8Z,11Z,14Z,17Z)/0:0)	M+H+K	209.123722	378.27701	0.0006856	Glycerolipids
HMDB35273	1-Acetoxy-2-hydroxy-5,12,15-heneicosatrien-4-one	M+H+K	209.123722	378.27701	0.0006856	Fatty acyls
HMDB36356	[12]-Gingerol	M+H+K	209.123722	378.27701	0.0006856	Benzene and substituted derivatives
HMDB11578	MG(20:4(5Z,8Z,11Z,14Z)/0:0/0:0)	M+H+K	209.123722	378.27701	0.0006856	Glycerolipids
HMDB36568	Persenone A	M+H+K	209.123722	378.27701	0.0006856	Not classified
HMDB07007	CPA(18:2(9Z,12Z)/0:0)	M+2H	209.123664	416.232775	0.0007436	Lineolic acid and derivatives
HMDB32890	2,6-Dimethoxy-4-phenanthrenol	M+2Na+H	209.123134	254.094294	0.0012736	Phenanthrenes and derivatives

Figure 4.92: Boxplot Urinary Variable ID 450 Post Surgery v Healthy Controls UHPLC- FTMS



p=2.66E-05

In this study urinary variable ID 450 is found to be increased in post surgery IBD patients compared to healthy controls. Metabolites considered biologically relevant are listed below.

MG(20:4(8Z,11Z,14Z,17Z)/0:0/0:0), MG(0:0/20:4(8Z,11Z,14Z,17Z)/0:0, MG(20:4(5Z,8Z,11Z,14Z)/0:0/0:0)) and 2-Arachidonylglycerol

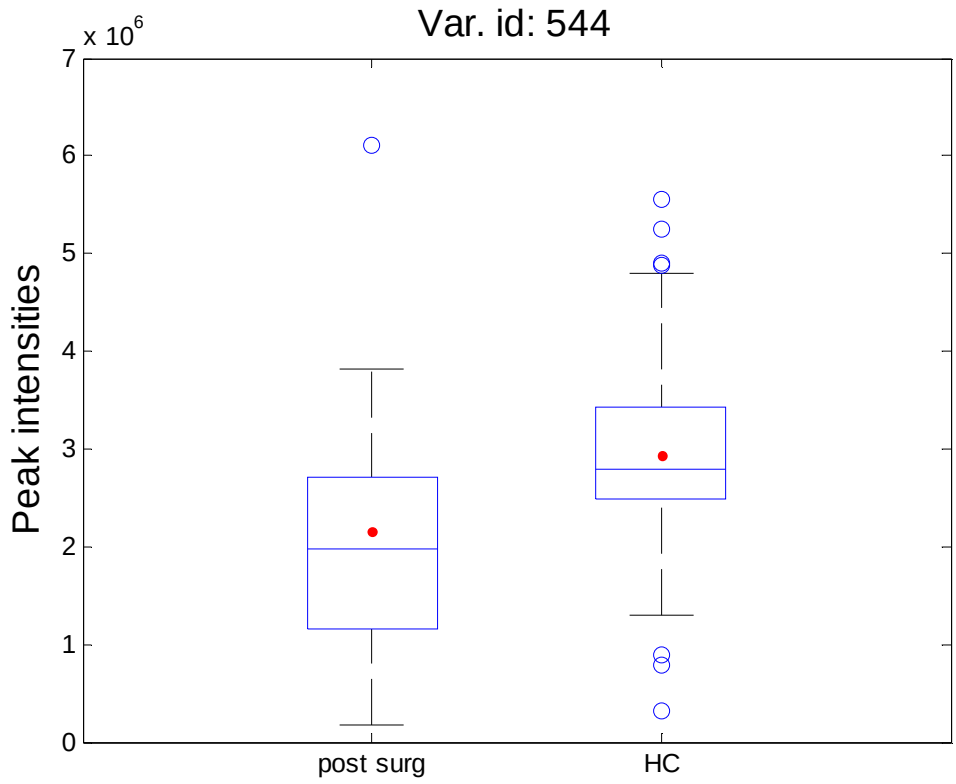
These metabolites are from the glycerolipid class. They are monoacylglycerides, a glyceride consisting of one fatty acid chain covalently bonded to a glycerol molecule through ester linkage. Monoacylglycerols are formed biochemically via release of a fatty acid from diacylglycerol by diacylglycerol lipase or hormone sensitive lipase.

Endocannabinoids, *N*-acylethanolamine *N*-arachidonoylglycerol and monacylglycerol 2-arachidonoylglycerol, are lipid mediators expressed in the GI tract. The endocannabinoid system is implicated in gut homeostasis, modulating gastrointestinal motility, visceral sensation, and inflammation, as well as being recently implicated in IBD pathogenesis (Alhouayek, Muccioli 2012). In our study these urinary metabolites are found to be raised in comparison to healthy controls. This may suggest that the endocannabinoid system is dysregulated post surgery IBD patients.

Table 4.51.3: Mass spectra search for 237.1222813m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB34671	(E)-5,8-Megastigmadien-4-one	M+2Na-H	237.122575	192.151415	0.0002937	Carbonyl compounds
HMDB36022	Isospirene	M+2Na-H	237.122575	192.151415	0.0002937	Dihydrofurans
HMDB29824	2,4-Diisopropyl-3-methylphenol	M+2Na-H	237.122575	192.151415	0.0002937	Benzene and substituted derivatives
HMDB35753	(R)-(E)-4,7-Megastigmadien-9-one	M+2Na-H	237.122575	192.151415	0.0002937	Prenol lipids
HMDB32541	4-(2,6,6-Trimethylcyclohex-1-enyl)but-2-en-4-one	M+2Na-H	237.122575	192.151415	0.0002937	Carbonyl compounds
HMDB36027	alpha-Damascone	M+2Na-H	237.122575	192.151415	0.0002937	Not classified
HMDB34959	Edulan I	M+2Na-H	237.122575	192.151415	0.0002937	Benzopyrans
HMDB29823	2,4-Diisopropyl-5-methylphenol	M+2Na-H	237.122575	192.151415	0.0002937	Prenol lipids
HMDB33545	(2E,4Z,7Z)-2,4,7-Tridecatrienal	M+2Na-H	237.122575	192.151415	0.0002937	Fatty acyls
HMDB29822	2,5-Diisopropyl-3-methylphenol	M+2Na-H	237.122575	192.151415	0.0002937	Prenol lipids

Figure 4.93: Boxplot Urinary Variable ID 544 Post Surgery v Healthy Controls UHPLC- FTMS



p=0.00029484

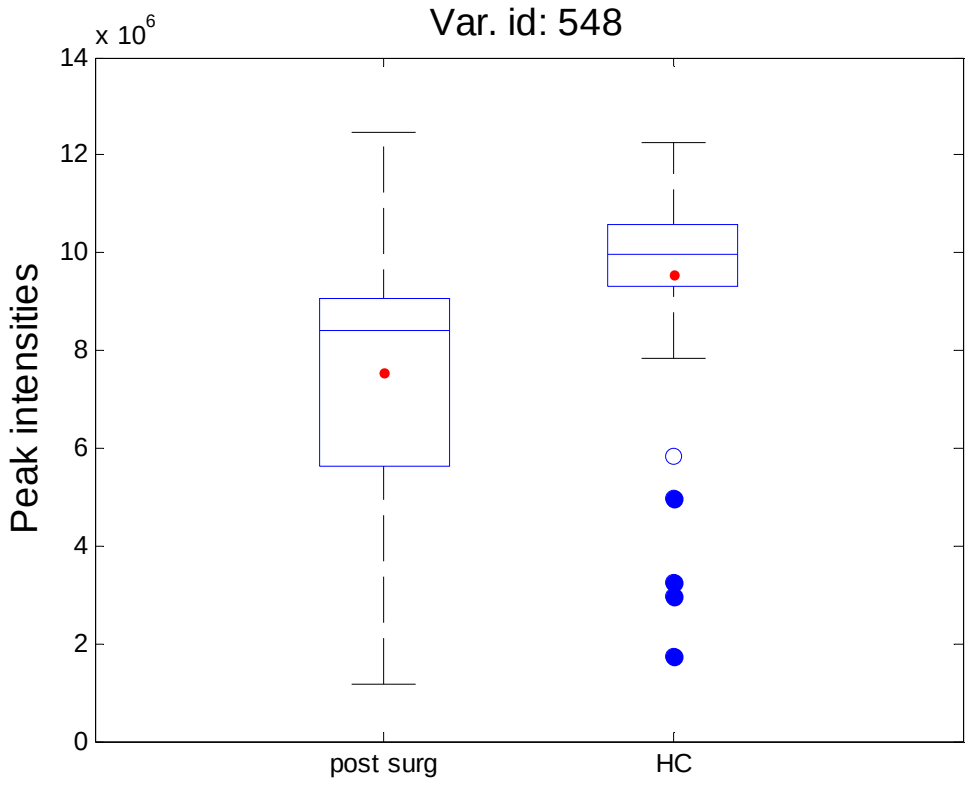
Urinary variable ID 544 is found to be reduced in post surgery IBD patients compared to healthy controls. All of the metabolites identified are dietary in nature. The findings may be reflective of post surgery IBD patients not yet returning to a diet comparable with healthy controls.

Table 4.51.4: Mass spectra search for 239.088045 m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB36131	S-Furanopetasitin	M+2Na	239.08774	432.197045	0.000305	Prenol lipids
HMDB40613	Kanzonol G	M+H+K	239.087336	438.204239	0.000709	Isoflavanoids
HMDB36597	1-Hydroxy-3,6,7-trimethoxy-2,8-diprenylxanthone	M+H+K	239.087336	438.204239	0.000709	Benzopyans
HMDB60791	6-Thioinosinic acid	M+2Na+H	239.086766	284.057926	0.001279	Carbohydrates and carbohydrate conjugates
HMDB612	6-	M+2Na+H	239.086766	284.057926	0.001279	Imidazopyri

69	Mercaptopurine riboside					midines
HMDB60897	Diphenhydramine N-glucuronide	M+2Na	239.090332	432.202227	0.002287	Carbohydrates and carbohydrate conjugates
HMDB33952	Gyromitrin	2M+K	239.090484	100.063663	0.002439	Hydrazines and derivatives
HMDB31642	N-Nitroso-pyrrolidine	2M+K	239.090484	100.063663	0.002439	Pyrrolidines
HMDB02511	3,4,5-Trimethoxy cinnamic acid	M+H	239.0914	238.084124	0.003355	Cinnamic acids and derivatives
HMDB31771	1-(2,4,5-Trimethoxy phenyl)-1,2-propanedione	M+H	239.0914	238.084124	0.003355	Benzene and substituted derivatives

Figure 4.94: Boxplot Urinary Variable ID 548 Post Surgery v Healthy Controls UHPLC- FTMS



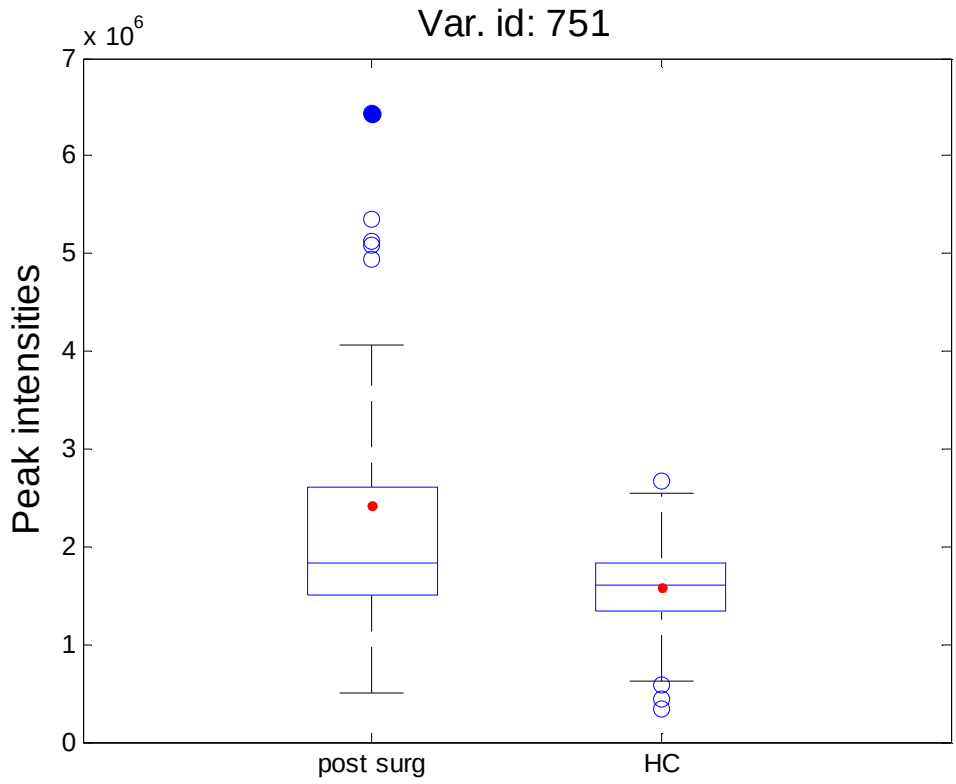
p=0.00063886

In this study urinary variable 548 is reduced in post surgery IBD patients compared to healthy controls. The metabolites identified are predominantly dietary in nature. The findings may be reflective of post surgery IBD patients not yet returning to a diet comparable with healthy controls.

Table 4.51.5: Mass spectra search for 303.2348021m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB15695	Roxatidine acetate	M+2Na+H	303.233747	348.204907	0.0010551	Piperidines
HMDB36875	Phytoene 1,2-epoxide	M+2Na	303.237076	560.495717	0.0022739	Prenol lipids
HMDB29854	1,2-Epoxy-1,2,7,7',8,8',11',12'-octahydro-psi,psi-carotene	M+2Na	303.237076	560.495717	0.0022739	Not classified
HMDB36775	Yucalexin A16	M+H	303.231856	302.22458	0.0029461	Prenol lipids
HMDB36295	Cycloartanyl ferulate	M+2H	303.231856	604.44916	0.0029461	Not classified
HMDB03598	Retinyl ester	M+H	303.231856	302.22458	0.0029461	Prenol lipids
HMDB39486	ent-8(17),13(16),14-Labdatrien-18-oic acid	M+H	303.231856	302.22458	0.0029461	Prenol lipids
HMDB36709	Yucalexin B14	M+H	303.231856	302.22458	0.0029461	Prenol lipids
HMDB15655	Methyltestosterone	M+H	303.231856	302.22458	0.0029461	Steroids and steroid derivatives
HMDB35692	8,15-Isopimaradien-18-oic acid	M+H	303.231856	302.22458	0.0029461	Prenol lipids

Figure 4.95: Boxplot Urinary Variable ID 751 Post Surgery v Healthy Controls UHPLC- FTMS



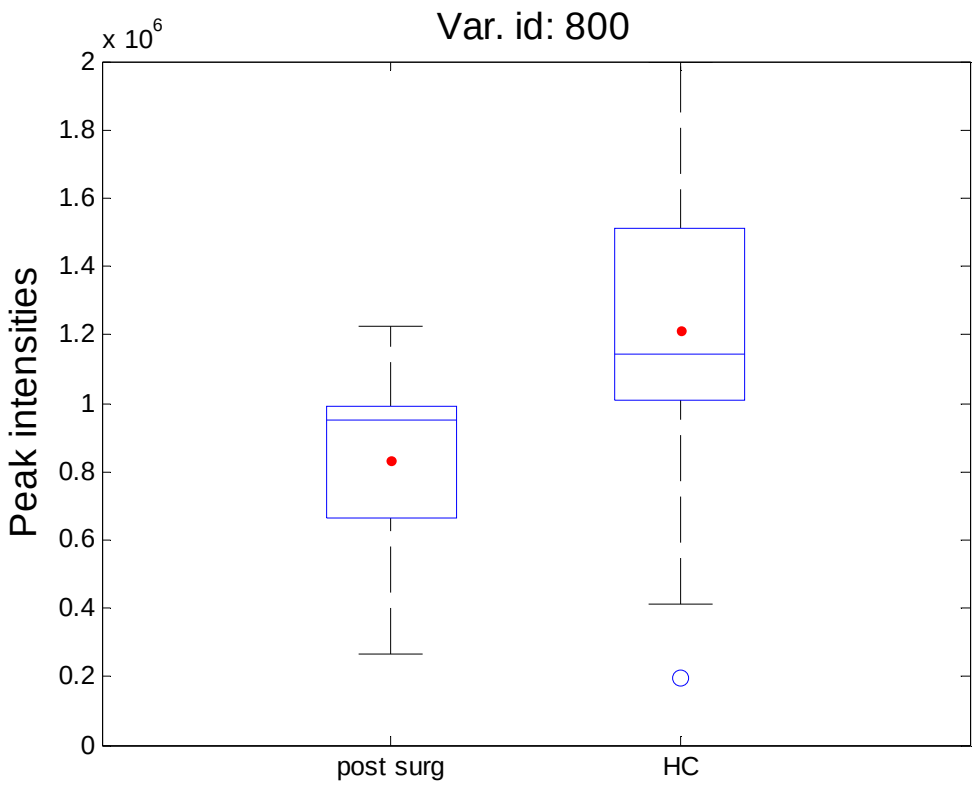
p=0.00040589

In this study urinary variable 751 is increased in post surgical IBD patients compared to healthy controls. Most of the variables identified are dietary in nature. Retinyl esther may be of biological interest.

Retinyl Ester

Retinyl ester, from the prenol lipid class, is the storage form of vitamin A. In IBD the absorption of vitamin A is normal, however serum levels have been shown to be reduced in active disease phases, returning to normal following treatment, withour supplementation (Janczewska, Bartnik et al. 1991). In our study we see increased urinary levels of retinyl ester in the post surgery IBD patients compared to healthy controls. This is difficult to explain, but may represent increased excretion following replenishment of body stores.

Figure 4.96: Boxplot Urinary Variable ID 800 Post Surgery v Healthy Controls UHPLC- FTMS



p=0.00012066

In this study urinary variable 800 is decreased in post surgical IBD patients compared to healthy controls. The metabolites for this variable ID have been identified and discussed previously.

4.16.2 Experiment 14.2: Metabolite Identification Post Surgery v Healthy Controls GC-ToF-MS

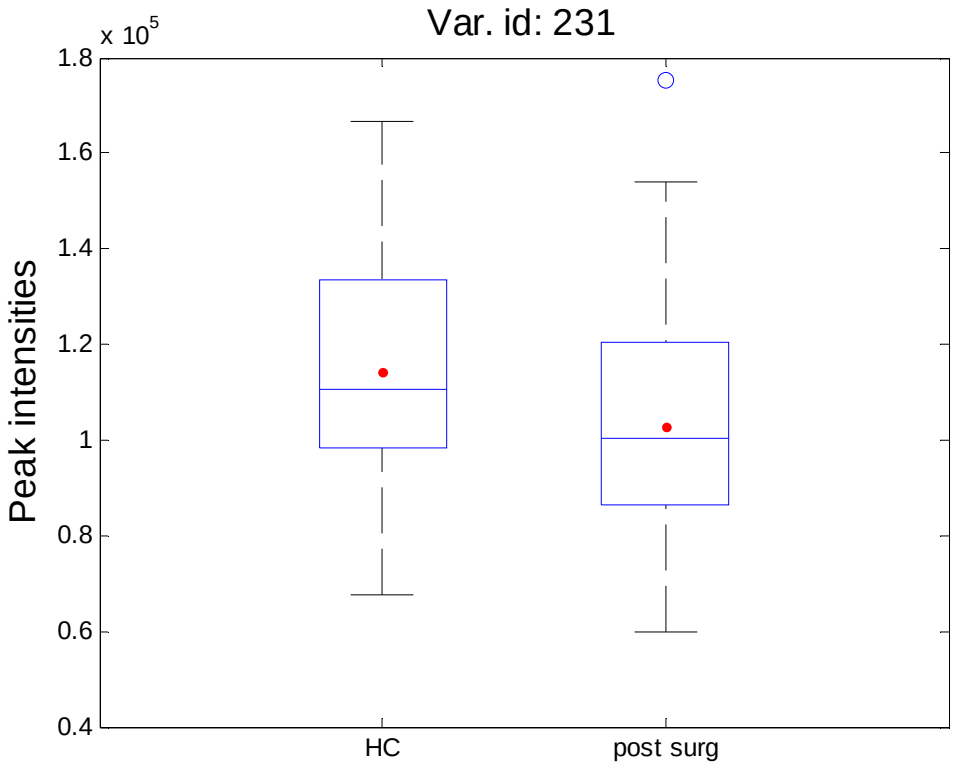
Table 4.52: Putative Metabolites Post Surgery v Healthy Controls GC-ToF-MS

Variable ID	Biofluid	Quant mass	Retention time	Putative match	p value	q value
82	Urine	77	915.078	Unknown	0.00076565	0.02220385
231	Urine	172	945.828	Uric acid - tetrakis	0.001411	0.040919
394	Urine	375	788.078	Unknown	0.0011832	0.0343128
401	Urine	442	946.478	Unknown	0.00011248	0.00326192
8	Serum	47	573.478	Unknown	6.80E-06	0.001122
14	Serum	49	577.428	Unknown	6.48E-05	0.010692
22	Serum	51	575.128	Unknown	0.00012231	0.02018115
27	Serum	52	571.628	Threonine or urea	7.98E-06	0.0013167
30	Serum	53	575.774	Unknown	1.32E-05	0.002178
34	Serum	54	577.453	Unknown	2.09E-05	0.0034485
45	Serum	56	574.878	Unknown	5.98E-06	0.0009867
64	Serum	61	575.528	Unknown	3.04E-05	0.005016
69	Serum	63	576.428	Unknown	6.81E-05	0.0112365
74	Serum	64	570.778	Urea	3.18E-06	0.0005247
76	Serum	65	571.903	Threonine or urea	1.26E-06	0.0002079
81	Serum	66	571.278	Threonine or urea	5.40E-07	8.91E-05
86	Serum	67	572.201	Unknown	6.39E-07	0.000105435
93	Serum	69	571.028	Threonine or urea	2.35E-06	0.00038775
98	Serum	70	561.694	Dihydroxybutanoic acid or Serine	3.07E-05	0.0050655
106	Serum	71	571.978	Threonine or urea	1.88E-06	0.0003102
107	Serum	72	572.378	Unknown	1.53E-06	0.00025245
121	Serum	75	574.929	Unknown	2.24E-06	0.0003696
133	Serum	78	572.928	Unknown	9.85E-06	0.00162525
137	Serum	79	570.426	Urea	1.98E-06	0.0003267
142	Serum	80	570.674	Urea	4.65E-06	0.00076725
157	Serum	84	575.428	Unknown	1.17E-05	0.0019305
177	Serum	89	355.368	Unknown	0.00028811	0.04753815
190	Serum	92	580.328	Unknown	0.0001573	0.0259545
218	Serum	99	570.728	Urea	6.91E-07	0.000114015
221	Serum	100	562.088	Dihydroxybutanoic acid or Serine	2.85E-06	0.00047025
228	Serum	102	563.626	Dihydroxybutanoic acid or Serine	0.00019559	0.03227235
266	Serum	111	571.579	Threonine or urea	1.69E-06	0.00027885
279	Serum	115	572.628	Unknown	1.70E-05	0.002805

291	Serum	117	578.178	Unknown	4.32E-05	0.007128
334	Serum	127	571.479	Threonine or urea	2.73E-06	0.00045045
355	Serum	132	576.428	Unknown	9.79E-06	0.00161535
383	Serum	139	570.378	Urea	9.24E-05	0.015246
389	Serum	140	574.228	Unknown	6.90E-05	0.011385
390	Serum	141	570.928	Urea	4.17E-06	0.00068805
428	Serum	151	580.351	Unknown	0.00023489	0.03875685
446	Serum	155	570.524	Urea	1.63E-06	0.00026895
455	Serum	157	570.478	Urea	1.72E-06	0.0002838
490	Serum	166	487.164	Glycerol	7.37E-11	1.22E-08
500	Serum	169	797.528	Citric acid	0.00025658	0.0423357
507	Serum	171	570.474	Urea	2.04E-06	0.0003366
509	Serum	172	570.678	Urea	1.91E-06	0.00031515
513	Serum	173	570.328	Urea	1.09E-06	0.00017985
563	Serum	186	570.728	Urea	8.37E-06	0.00138105
569	Serum	187	571.628	Threonine or urea	7.30E-06	0.0012045
574	Serum	189	570.078	Urea	3.73E-07	6.15E-05
576	Serum	190	569.928	Urea	5.27E-07	8.70E-05
582	Serum	191	569.826	Urea	2.74E-07	4.52E-05
637	Serum	204	568.779	Urea	0.00011918	0.0196647
990	Serum	318	829.026	Glucuronic acid	7.71E-05	0.0127215
1325	Serum	432	841.678	myo-inositol	0.00017877	0.02949705

The metabolites previously discussed are not repeated. Biologically relevant metabolites are considered below.

Figure 4.97: Boxplot Urinary Variable ID 231Uric Acid Post Surgery v Healthy Controls GC-ToF-MS

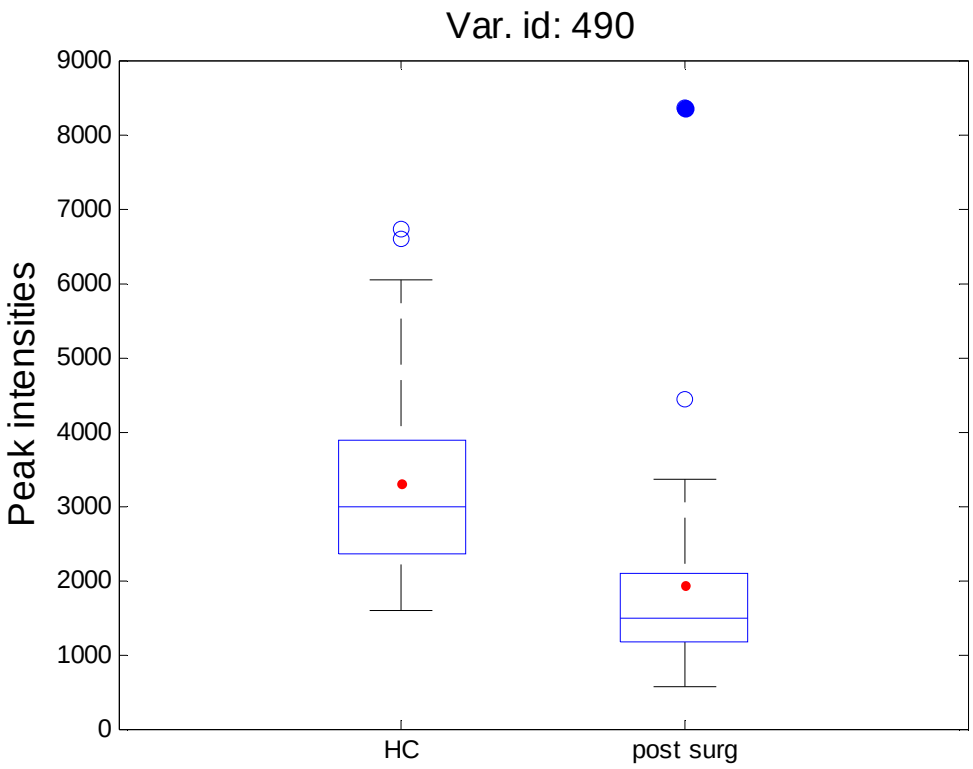


p=0.001411

Uric acid

The variable ID 231, identified as uric acid is reduced in the urine of post surgery IBD patients compared to healthy controls. Uric acid is a heterocyclic purine derivative that is the final oxidation product of purine metabolism. Thiopurines are known to induce and maintain remission of IBD, and the methyl thioinosine monophosphate / 6-thioguanine nucleotide concentration ratio has been associated with drug efficacy. Most genes that correlate with thiopurine metabolite levels have been shown to correlate with disease activity or to participate in networks with candidate IBD susceptibility genes involved in processes such as purine metabolism, cytokine signaling, and functioning of invariant natural killer T cells, T cells and B cells (Haglund, Almer et al. 2013). Interestingly, levels of uric acid in the urine of post surgery IBD patients are lower than in healthy controls. This may be due to reduced excretion as IBD patients are more likely to be taking thiopurine medications than HCs.

Figure 4.98: Boxplot Serum Variable ID 490 Glycerol Post Surgery v Healthy Controls GC-ToF-MS



p=7.37E-11

Glycerol

The variable ID identified as glycerol is reduced in the serum of post surgery IBD patients compared to healthy controls. Glycerol is an important component of triglycerides and of phospholipids. It belongs to the class of organic compounds known as sugar alcohols. When the body uses stored fat as a source of energy, glycerol and fatty acids are released into the bloodstream. The glycerol component can be converted to glucose by the liver and provides energy for cellular metabolism. Elevated glycerol levels have been identified in the faeces of CD when compared to both UC and HC (Marchesi J.R., Holmes E. et al. 2007). The reason for this is unknown. In our study we see reduced levels of glycerol in the serum of post surgery IBD patients when compared to HCs. This has never been investigated in IBD patients, however, in patients undergoing abdominal hysterectomy, increased rate of glycerol turnover has been noted postoperatively, and increased glycerol concentration in the perioperative period (Schricker, Berroth et al. 1997). Our samples were taken 8 weeks post operatively and therefore perioperative changes should not be relevant. This result is unexplained.

4.16.3 Experiment 14 Summary

Urinary metabolites belonging to the super class alkaloids and derivatives, and the classes organic disulphides, benzene and substituted derivatives, carboxylic acids and derivatives, azoles, and 2-arylbenzofuran flavonoids are reduced in post surgical IBD patients when compared to healthy controls. Metabolites in the class prenol lipids and glycerolipids are increased in the urine of post surgical IBD patients when compared to healthy controls.

4.17 Experiment 15: Metabolite Identification Treatment Naïve v Post Treatment (Paired)

In this experiment we aim to identify metabolites that allow differentiation between treatment naïve IBD patients, and those who have undergone treatment.

Table 4.53 Experiment 15 number of samples analysed

	Treatment Naive	Post Treatment
Serum samples	6	110
Urine samples	6	109

4.17.1 Experiment 15.1: Metabolite Identification Treatment Naïve v Post Treatment (Paired) UHPLC-FTMS

Table 4.54: Important Variables Identified Treatment Naïve v Post Treatment (Paired) UHPLC-FTMS

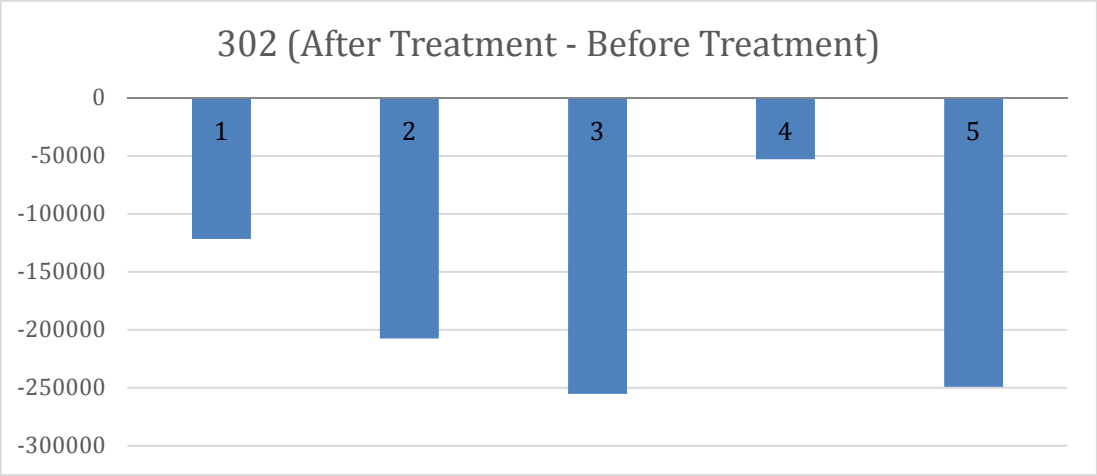
Variable ID	Biofluid	M/z	Retention time	p value	q value
302	Serum	272.9491301	45.6825	0.0014647	0.0497998

Table 4.54.1: Mass spectra search for 272.9491301 m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB14026	p-Chlorobenzene sulfonylurea	M+K	272.949748	233.98659	0.0006179	Benzene and substituted derivatives
HMDB01187	1D-Myo-inositol 1,3,4,6-tetrakisphosphate	M+2Na	272.953573	499.92871	0.0044429	Alcohols and polyols
HMDB01059	Inositol 1,3,4,5-tetraphosphate	M+2Na	272.953573	499.92871	0.0044429	Alcohols and polyols
HMDB04527	1D-Myo-inositol 1,4,5,6-tetrakisphosphate	M+2Na	272.953573	499.92871	0.0044429	Alcohols and polyols
HMDB03848	D-Myo-inositol 3,4,5,6-tetrakisphosphate	M+2Na	272.953573	499.92871	0.0044429	Alcohols and polyols
HMDB37638	Ammonium peroxydisulfate	M+2Na-H	272.943367	227.972207	0.0057631	Non-metal oxoanionic compounds

HMDB30800	3-Methoxy-4,5-methylenedioxybenzoic acid	M+2K+H	272.956213	196.037173	0.0070829	Benzene and substituted derivatives
HMDB59809	4-Hydroxy-benzenepropanedioate	M+2K+H	272.956213	196.037173	0.0070829	Benzene and substituted derivatives
HMDB15265	Tiludronate	M+2Na+H	272.957199	317.928359	0.0080689	Organic phosphonic acids and derivatives
HMDB32856	3,3',4,4'-Tetrachloroazobenzene	M+2Na+H	272.957349	317.928509	0.0082189	Azobenzenes

Figure 4.99: Barchart Serum Variable ID 302 UHPLC-FTMS paired sample comparison of treatment naïve IBD patients post and pre treatment



p=0.0014647

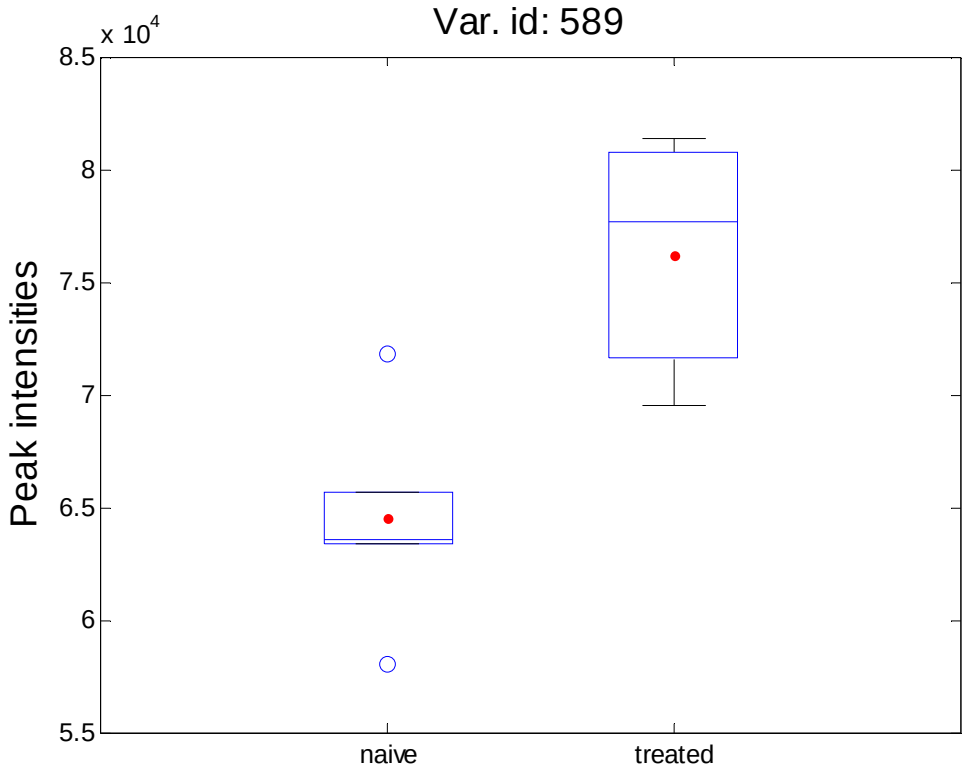
Variable ID 302 shows reduced serum levels post treatment when compared patients are compared to their pre treatment drug naïve samples. All of the metabolites identified are dietary or drug related, and are not biologically relevant in IBD.

4.17.2 Experiment 15.2: Metabolite Identification Treatment Naïve v Post Treatment (Grouped Results) GC-ToF-MS

Table 4.55: Putative Metabolites Table 4.41: Putative Metabolites Treatment Naïve v Post Treatment (Grouped Results) GC-ToF-MS

Variable ID	Biofluid	Quant mass	Retention time	Putative match	p value	q value
589	Serum	193	545.928	Phosphate	0.0001098	0.014823

Figure 4.100: Boxplot Serum Variable ID 589 Phosphate Treatment Naïve v Post Treatment (Grouped Results) GC-ToF-MS



p=0.0001098

In this experiment, the serum variable ID 589 identified as phosphate is lower in treatment naïve patients than in those who have been commenced on treatment for IBD.

Phosphate

Phosphate is from the class non-metal oxoanionic compounds. Hypophosphataemia is associated with low albumin, malnutrition and refeeding syndrome (Imel, Econs 2012). In our study, serum phosphate was reduced in drug naïve IBD patients and improved in the same patients following treatment.

4.17.3 Experiment 15 Summary

Metabolites in the class non-metal oxoanionic compounds were found to be increased in the serum of drug naïve IBD patients pre treatment compared to post treatment.

4.18 Experiment 16: Metabolite Identification Treatment Naïve v Healthy Controls

In this experiment we aim to identify metabolites differentiating between treatment naïve IBD patients and HCs.

Table 4.56 Experiment 16 number of samples analysed

	Treatment Naive	Healthy Controls
Serum samples	6	62
Urine samples	28	60

4.18.1 Experiment 16.1: Metabolite Identification Treatment Naïve v Healthy Controls UHPLC-FTMS

Table 4.57: Important Variables Identified Treatment Naïve v Healthy Controls UHPLC-FTMS

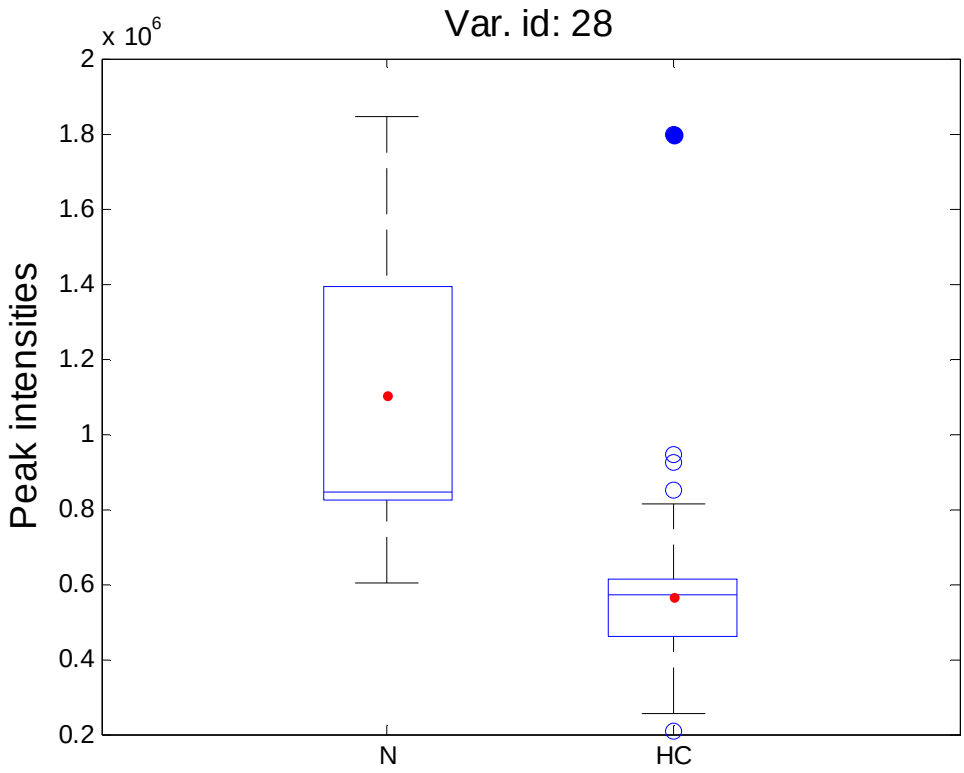
Variable ID	Biofluid	M/z	Retention time	p value	q value
28	Serum	124.9759098	67.42955	0.00036218	0.00796796
493	Serum	386.7679997	992.1447	0.0021199	0.0466378
529	Serum	404.2888825	998.1519	0.00013426	0.00295372
12	Urine	106.0362854	240.0221	0.00011055	0.01006005
75	Urine	130.0491589	291.87825	0.00018568	0.01689688
212	Urine	161.0399971	822.8008	4.27E-05	0.0038857
214	Urine	161.0799605	276.0668	1.07E-06	9.74E-05
265	Urine	171.0758451	50.5109	0.0002354	0.0214214
373	Urine	194.9948803	1269.253	0.00015633	0.01422603
501	Urine	224.0294257	244.16795	4.60E-05	0.004186
680	Urine	279.1330075	177.2557	5.31E-05	0.0048321
706	Urine	287.099141	258.5507	0.00019558	0.01779778
800	Urine	334.0909909	299.601	1.08E-05	0.0009828

Table 4.57.1: Mass spectra search for 124.9759098 m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB31332	Chloromethyl methyl ether	M+2Na-H	124.974052	80.002892	0.0018578	Ethers
HMDB60363	2,5-Dichloro-4-oxohex-2-enedioate	M+H+Na	124.970036	225.943579	0.0058738	Keto acids and derivatives
HMDB40362	(±)-Glycerol 1-monophosphate K salt (1:2)	M+2H	124.969995	247.925438	0.0059148	Not classified
HMDB33875	2,3-Dihydrothiophene	M+K	124.982179	86.019021	0.0062692	Dihydrothiophenes
HMDB339	Divinyl	M+K	124.982179	86.019021	0.0062692	Thioethers

22	sulfide					
HMDB41967	Oltipraz	M+H+Na	124.982902	225.96931	0.0069922	Diazines
HMDB02179	Peroxynitrite	2M+H	124.982912	61.987818	0.0070022	Non-metal oxoanionic compounds
HMDB33202	Di-2-propenyl tetrasulfide	M+H+K	124.968484	209.966533	0.0074258	Sulfenyl compounds

Figure 4.101: Boxplot Serum Variable ID 28 Treatment Naïve v Healthy Controls UHPLC-FTMS



p=0.00036218

In this experiment, peak intensities in serum variable ID 28 are higher in treatment naïve IBD patients than in healthy controls. Peroxynitrite is a metabolite of biological interest. The rest of the metabolites identified are dietary.

Peroxynitrite

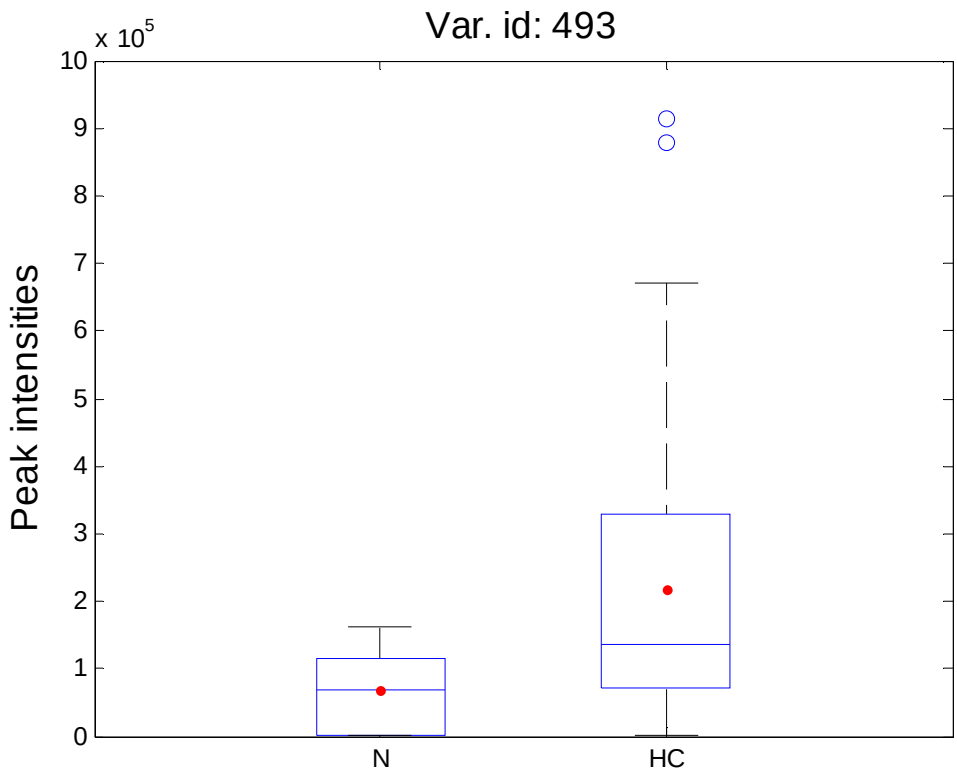
Peroxynitrite belongs to the class of inorganic compounds known as non-metal peroxynitrites and is a potent oxidant synthesised by the cell during its normal metabolism. The peroxynitrite anion (ONOO-) is a reactive species produced in the reaction between the superoxide anion (O2*-) and nitric oxide (*NO). Peroxynitrite (ONOO-) is a strong oxidation and nitration agent, which damages DNA, proteins and other cellular structures. ONOO- is involved in several pathological conditions such as

inflammation, arteriosclerosis, and neurodegenerative and cardiovascular disorders. The immunoreactivity of both peroxynitrite-mediated protein nitration and nitric oxide synthase are significantly higher in UC than in CD (Kruidenier, Kuiper et al. 2003).

Table 4.57.2: Mass spectra search for 386.7679997 m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB35990	Cerebroside B	M+2Na	386.769135	727.559833	0.0011353	Sphingolipids
HMDB00564	PC(16:0/16:0)	M+H+K	386.766295	733.562155	0.0017047	Glycerophospholipids
HMDB08031	PC(18:0/14:0)	M+H+K	386.766295	733.562155	0.0017047	Glycerophospholipids
HMDB07871	PC(14:0/18:0)	M+H+K	386.766295	733.562155	0.0017047	Glycerophospholipids
HMDB09219	PE(20:0/15:0)	M+H+K	386.766295	733.562155	0.0017047	Glycerophospholipids
HMDB08899	PE(15:0/20:0)	M+H+K	386.766295	733.562155	0.0017047	Glycerophospholipids
HMDB11359	PE(P-16:0/22:5(4Z,7Z,10Z,13Z,16Z))	M+H+Na	386.766217	749.53594	0.0017827	Glycerophospholipids
HMDB09447	PE(20:4(8Z,11Z,14Z,17Z)/P-18:1(9Z))	M+H+Na	386.766217	749.53594	0.0017827	Glycerophospholipids
HMDB09642	PE(22:5(4Z,7Z,10Z,13Z,16Z)/P-16:0)	M+H+Na	386.766217	749.53594	0.0017827	Glycerophospholipids
HMDB11451	PE(P-18:1(9Z)/20:4(5Z,8Z,11Z,14Z))	M+H+Na	386.766217	749.53594	0.0017827	Glycerophospholipids

Figure 4.102: Boxplot Serum Variable ID 493 Treatment Naïve v Healthy Controls UHPLC-FTMS



p=0.0021199

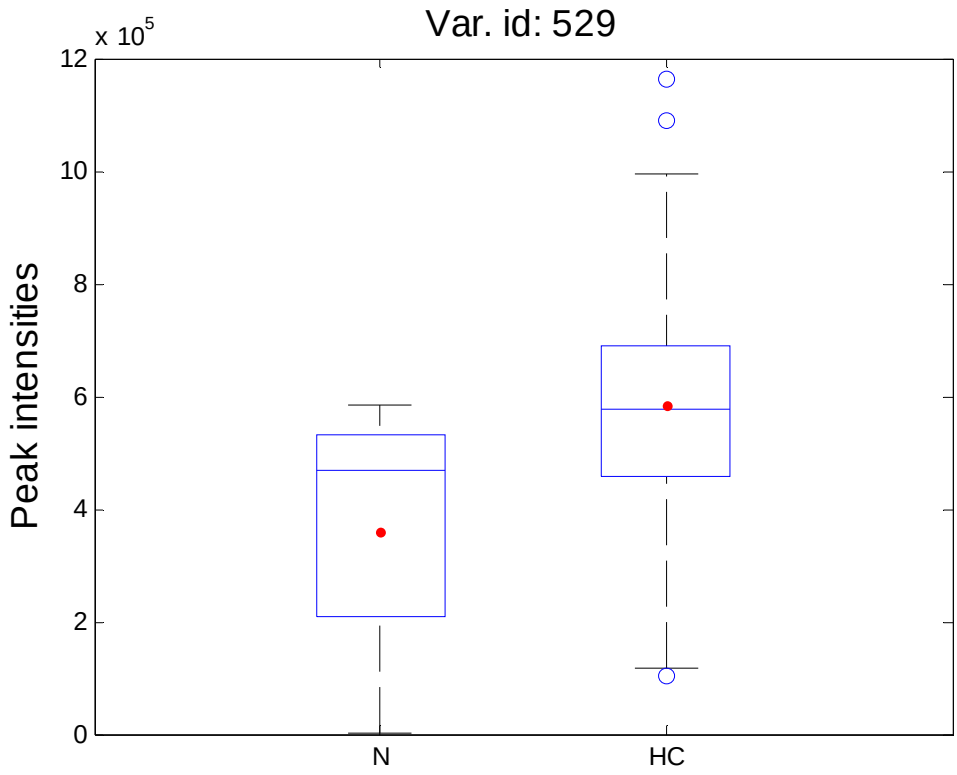
In this experiment, peak intensities of serum variable ID 493 were lower in treatment naïve IBD patients than in HCs. All of the metabolites identified are dietary in nature.

Table 4.57.3: Mass spectra search for 404.2888825 m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB35516	5-Decanoyl-2-nonylpyridine	M+2Na-H	404.289975	359.318815	0.0010925	Carbonyl compound
HMDB47901	TG(14:1(9Z)/14:1(9Z)/18:3(9Z,12Z,15Z))	M+H+K	404.298612	768.62679	0.0097295	Not classified
HMDB42295	TG(14:0/14:1(9Z)/18:4(6Z,9Z,12Z,15Z))	M+H+K	404.298612	768.62679	0.0097295	Not classified
HMDB48253	TG(14:1(9Z)/18:3(9Z,12Z,15Z)/14:1(9Z))	M+H+K	404.298612	768.62679	0.0097295	Not classified
HMDB427	TG(14:0/18	M+H+K	404.298612	768.62679	0.0097295	Not

88	:4(6Z,9Z,12Z,15Z)/14:1(9Z))					classified
HMDB47741	TG(14:1(9Z)/14:0/18:4(6Z,9Z,12Z,15Z))	M+H+K	404.298612	768.62679	0.0097295	Not classified
HMDB47894	TG(14:1(9Z)/14:1(9Z)/18:3(6Z,9Z,12Z))	M+H+K	404.298612	768.62679	0.0097295	Not classified
HMDB48092	TG(14:1(9Z)/18:3(6Z,9Z,12Z)/14:1(9Z))	M+H+K	404.298612	768.62679	0.0097295	Not classified

Figure 4.103: Boxplot Serum Variable ID 529 Treatment Naïve v Healthy Controls UHPLC-FTMS



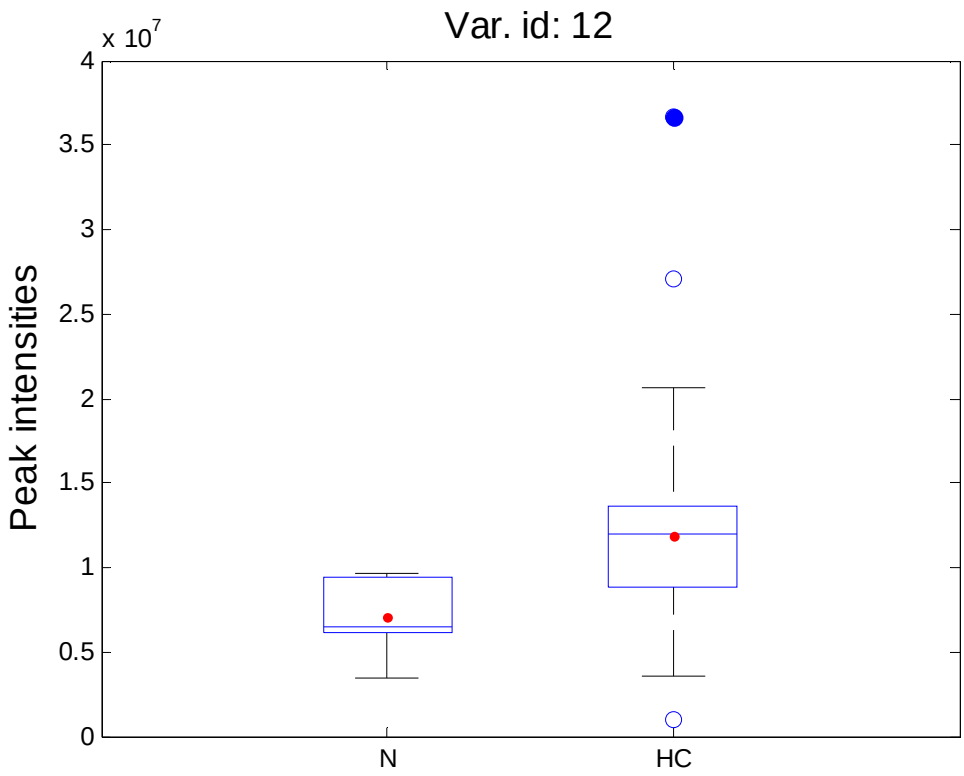
p=0.00013426

In this experiment, peak intensities of serum variable ID 529 were lower in treatment naïve IBD patients than in HCs. All of the metabolites identified are dietary in nature.

Table 4.57.4: Mass spectra search for 106.0362854m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB15126	Oxaprozin	M+2H+Na	106.036321	293.105193	0.0000356	Azoles
HMDB33048	Ethyl 1-(propylthio) propyl disulfide	M+2H	106.035807	210.057063	0.0004784	Organic disulfides
HMDB33049	Butyl 1-(methylthio)propyl disulfide	M+2H	106.035807	210.057063	0.0004784	Organic disulfides
HMDB15369	Chlorprothixene	M+3H	106.035559	315.084848	0.0007264	Benzothiopyrans
HMDB12488	1,2,3,4-Tetrahydro-beta-carboline	M+H+K	106.035241	172.100048	0.0010444	Indoles and derivatives
HMDB03929	5-Aminoimidazole	M+Na	106.037565	83.048347	0.0012796	Azoles
HMDB29862	Cyromazine	M+2Na	106.037565	166.096694	0.0012796	Triazines
HMDB40578	4-Thiocyanatophenol	M+2Na+H	106.038024	151.009184	0.0017386	Benzene and substituted derivatives
HMDB34413	1,2-Benzisothiazol-3(2H)-one	M+2Na+H	106.038024	151.009184	0.0017386	Benzothiazoles
HMDB06029	N-Acetylglutamine	M+H+Na	106.0381	188.079707	0.0018146	Carboxylic acids and derivatives

Figure 4.104: Boxplot Urinary Variable ID 12 Treatment Naïve v Healthy Controls UHPLC-FTMS



p=0.00011055

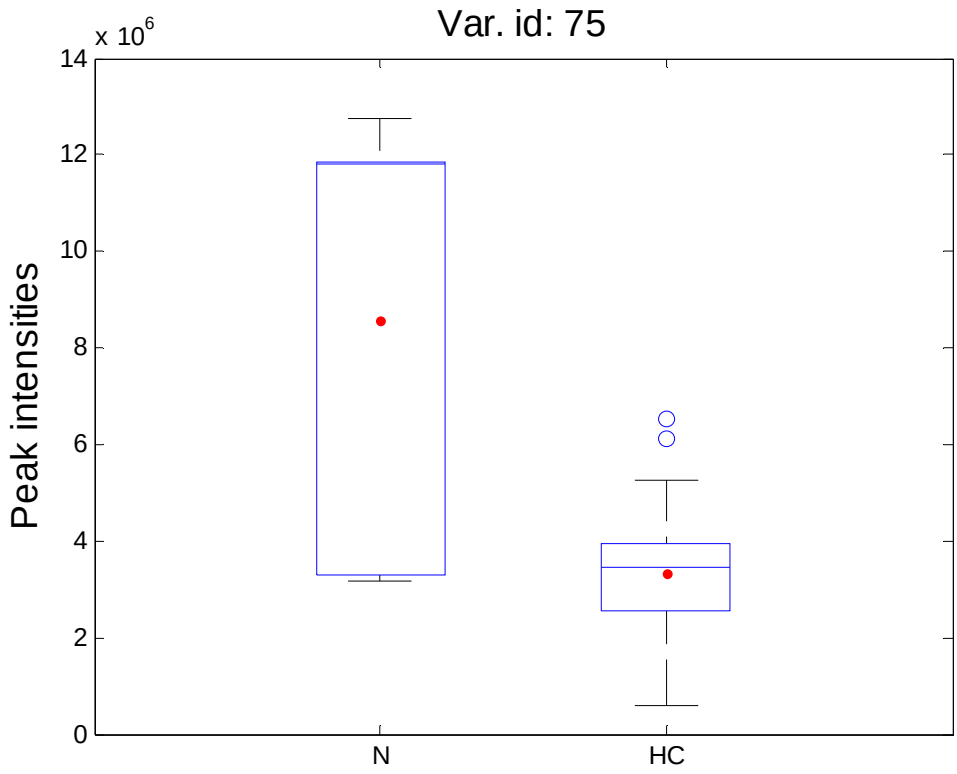
In this experiment, lower peak intensities of urinary variable ID 12 were seen in treatment naïve IBD patients compared to HCs. These metabolites have been discussed previously.

Table 4.57.5: Mass spectra search for 130.0491589 m/z

Compound	**Name**	**Adduct**	**Adduct MW (Da)**	**Compound MW (Da)**	**Delta**	**Class**
HMDB06239	S-aminomethyldihydrolipoamide	M+H+Na	130.049099	236.101705	0.0000599	Fatty acyls
HMDB28989	Phenylalanyl-Arginine	M+3Na	130.049248	321.18009	0.0000891	Not classified
HMDB28716	Arginyl-Phenylalanine	M+3Na	130.049248	321.18009	0.0000891	Carboxylic acids and derivatives
HMDB30986	2-Carboxy-5,7-dimethyl-4-octanolide	M+2Na	130.049473	214.120509	0.0003141	Lactones
HMDB30985	Alpha-Carboxy-delta-decalactone	M+2Na	130.049473	214.120509	0.0003141	Lactones

HMDB00805	Pyrrolidonecarboxylic acid	M+H	130.049869	129.042593	0.0007101	Carboxylic acids and derivatives
HMDB01843	N-Acryloylglycine	M+H	130.049869	129.042593	0.0007101	Carboxylic acids and derivatives
HMDB61093	Dimethadione	M+H	130.049869	129.042593	0.0007101	Azolines
HMDB02331	Imidazoleacetic acid riboside	M+2H	130.049869	258.085186	0.0007101	Imidazole ribonucleosides and ribonucleotides
HMDB00267	Pyroglutamic acid	M+H	130.049869	129.042593	0.0007101	Carboxylic acids and derivatives

Figure 4.105: Boxplot Urinary Variable ID 75 Treatment Naïve v Healthy Controls UHPLC-FTMS



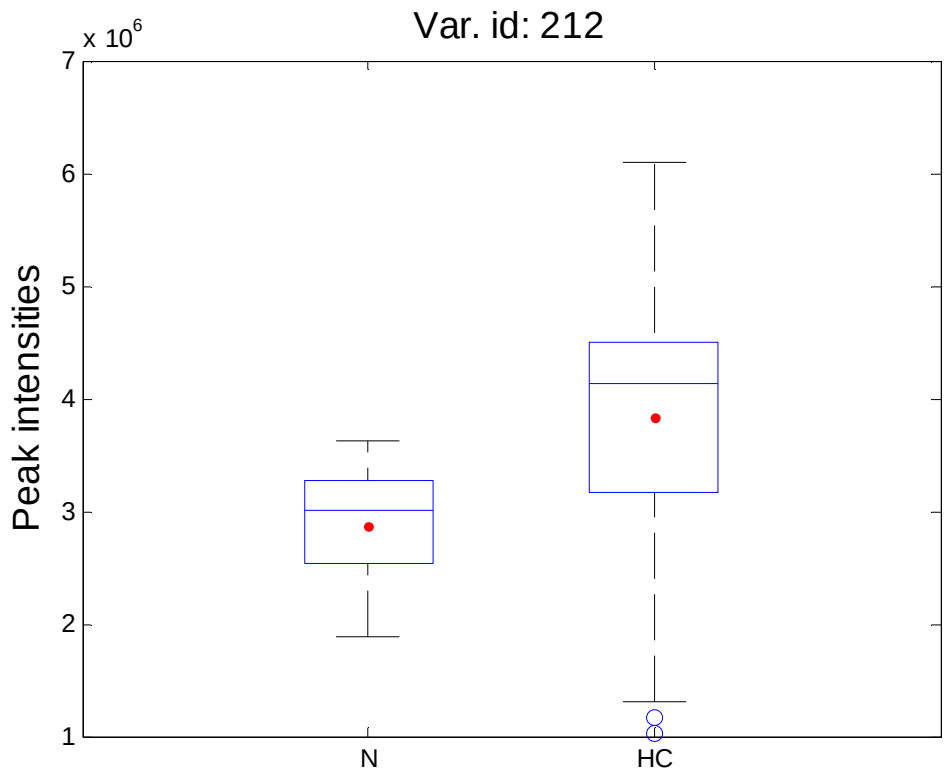
p=0.00018568

In this experiment, higher peak intensities of urinary variable ID 75 were seen in treatment naïve IBD patients compared to HCs. These metabolites have been discussed previously.

Table 4.57.6: Mass spectra search for 161.0399971 m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB32600	Ptelatoside A	M+3Na	161.040084	414.152597	0.0000869	Not classified
HMDB29512	4',5-Dihydroxy-7-methoxy-6-methylflavone	M+H+Na	161.040309	298.084124	0.0003119	Flavonoids
HMDB38811	Alfalone	M+H+Na	161.040309	298.084124	0.0003119	Isoflavonoids
HMDB30718	Sayanedin	M+H+Na	161.040309	298.084124	0.0003119	Isoflavonoids
HMDB31314	Benzyl methyl sulfide	M+Na	161.039539	138.050321	0.0004581	Benzene and substituted derivates
HMDB41361	2-Ethylbenzenethiol	M+Na	161.039539	138.050321	0.0004581	Benzene and substituted derivates
HMDB32467	(+/-)-1-Phenylethyl mercaptan	M+Na	161.039539	138.050321	0.0004581	Benzene and substituted derivates
HMDB32019	2,6-Dimethylbenzenethiol	M+Na	161.039539	138.050321	0.0004581	Benzene and substituted derivates
HMDB30791	Dihydromethysticin	M+2Na	161.039105	276.099774	0.0008921	Not classified
HMDB30145	Ascochitine	M+2Na	161.039105	276.099774	0.0008921	Benzene and substituted derivates

Figure 4.106: Boxplot Urinary Variable ID 212 Treatment Naïve v Healthy Controls UHPLC-FTMS



p=4.27E-05

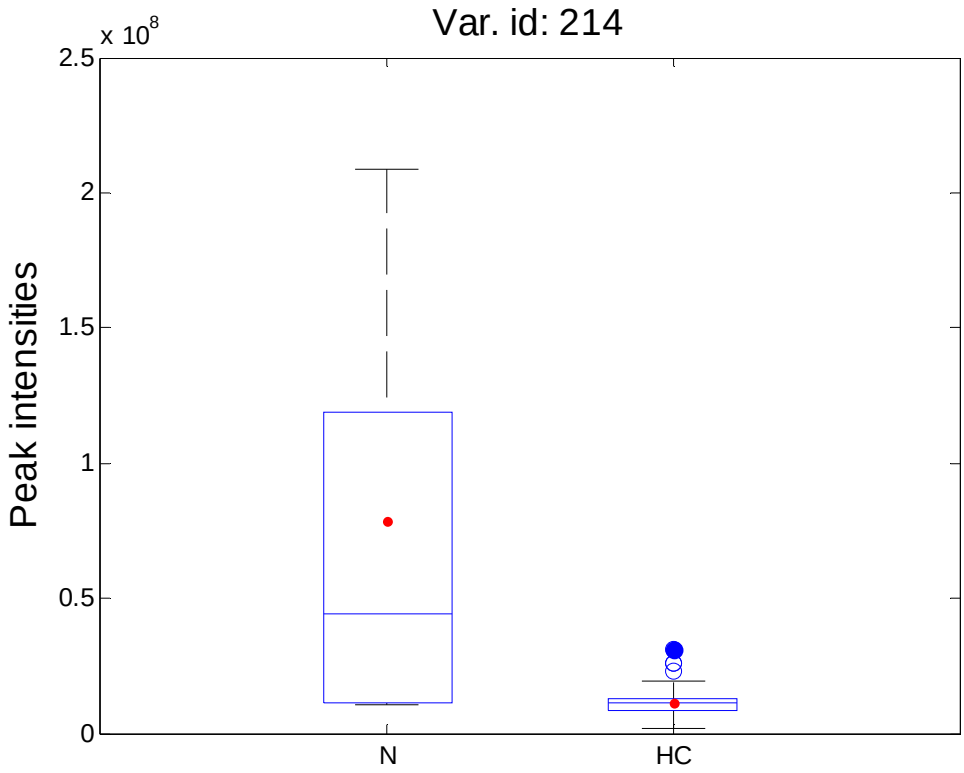
In this experiment, lower peak intensities of urinary variable ID 212 were seen in treatment naïve IBD patients compared to HCs. All of the metabolites identified are dietary in nature.

Table 4.57.7: Mass spectral search for 161.0799605 m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB36187	2-[(2-Furanylmethyl)thio]-6-methylpyrazine	M+2Na+H	161.080224	206.051384	0.0002635	Thioethers
HMDB32414	2-Methyl-3 or 5 or 6-(furfurylthio)pyrazine (mixture of isomers)	M+2Na+H	161.080224	206.051384	0.0002635	Thioethers
HMDB02504	3-Sulfodeoxycholic acid	M+2H+Na	161.079198	458.233824	0.0007625	Steroids and steroid deivatives
HMDB59722	Mono-methyl-adipate	M+H	161.080835	160.073559	0.0008745	Fatty acyls
HMDB608	Valpronic	M+2H	161.080835	320.147118	0.0008745	O-

89	acid beta-O-glucuronide					glucuronides
HMDB00555	3-Methyladipic acid	M+H	161.080835	160.073559	0.0008745	Fatty acyls
HMDB59738	2-Ethylglutaric acid	M+H	161.080835	160.073559	0.0008745	Not classified
HMDB00901	Valproic acid glucuronide	M+2H	161.080835	320.147118	0.0008745	Carbohydrates and carbohydrate conjugates
HMDB00857	Pimelic acid	M+H	161.080835	160.073559	0.0008745	Fatty acyls
HMDB61239	2-Propylsuccinic acid	M+H	161.080835	160.073559	0.0008745	Not classified

Figure 4.107: Boxplot Urinary Variable ID 214 Treatment Naïve v Healthy Controls UHPLC-FTMS



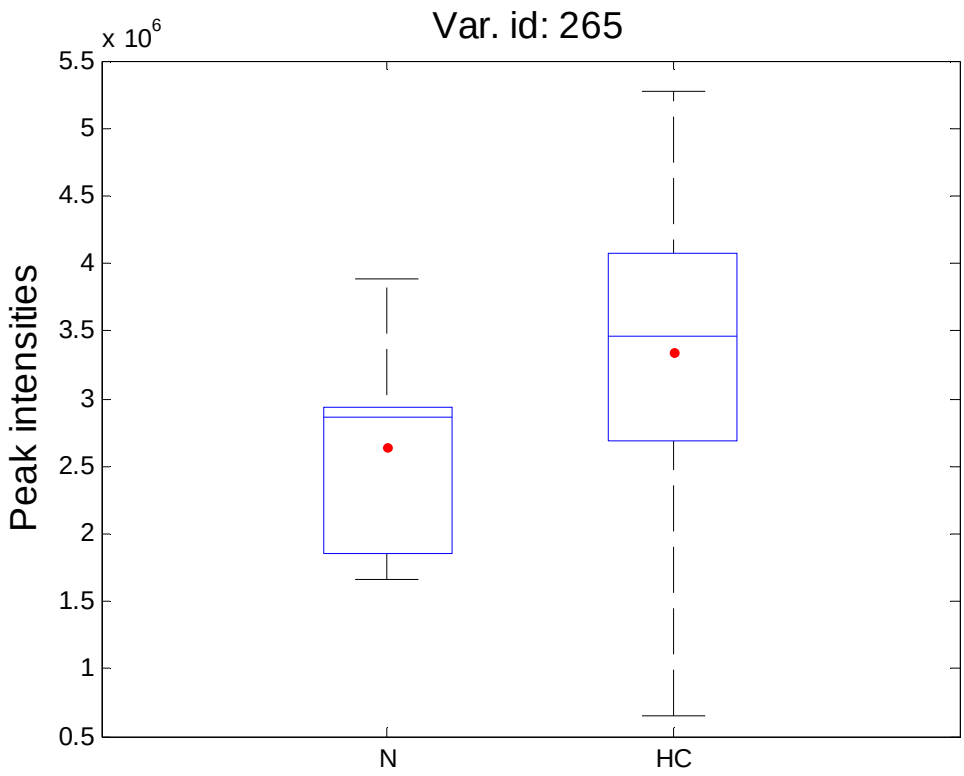
p=1.07E-06

In this experiment, higher peak intensities of urinary variable ID 214 were seen in treatment naïve IBD patients compared to HCs. None of the metabolites identified are biologically relevant.

Table 4.57.8: Mass spectra search for 171.0758451 m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB14322	Fluvoxamine	M+H+Na	171.076003	318.155513	0.0001579	Benzene and substituted derivatives
HMDB35242	(S,E)-Filbertone	M+2Na-H	171.075625	126.104465	0.0002201	Not classified
HMDB30966	(3xi,5Z)-1,5-Octadien-3-ol	M+2Na-H	171.075625	126.104465	0.0002201	Fatty acyls
HMDB39769	6-Octenal	M+2Na-H	171.075625	126.104465	0.0002201	Carbonyl compounds
HMDB31301	2-Octen-4-one	M+2Na-H	171.075625	126.104465	0.0002201	Carbonyl compounds
HMDB30961	2-Octenal	M+2Na-H	171.075625	126.104465	0.0002201	Carbonyl compounds
HMDB35390	5-Octen-2-one	M+2Na-H	171.075625	126.104465	0.0002201	Carbonyl compounds
HMDB31591	5-Methyl-5-hepten-2-one	M+2Na-H	171.075625	126.104465	0.0002201	Carbonyl compounds
HMDB31197	2,4,4-Trimethylcyclopentanone	M+2Na-H	171.075625	126.104465	0.0002201	Carbonyl compounds
HMDB35915	Sulcatone	M+2Na-H	171.075625	126.104465	0.0002201	Carbonyl compounds

Figure 4.108: Boxplot Urinary Variable ID 265 Treatment Naïve v Healthy Controls UHPLC-FTMS



p=0.0002354

In this experiment, peak intensities of urinary variable 265 are lower in treatment naïve IBD patients than in HCs. All of the metabolites identified are dietary or medication related. However, fluvoxamine is of interest.

Fluvoxamine

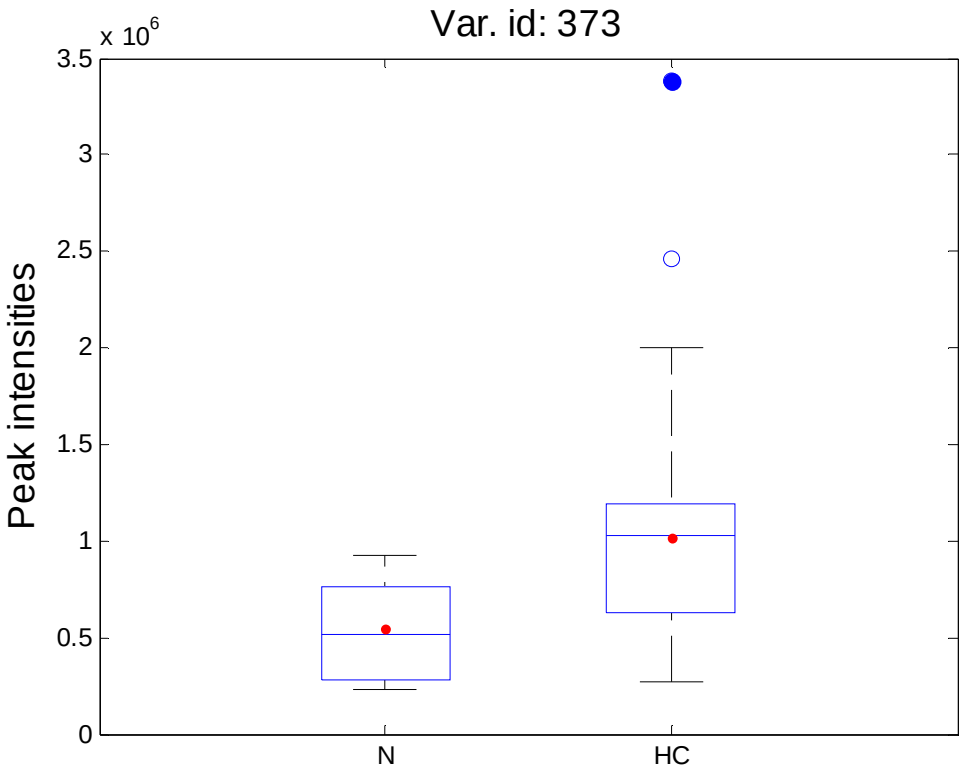
Fluvoxamine is a selective serotonin reuptake inhibitor antidepressant medication. Recently, its anti-inflammatory and anti-colitic effects have been shown in colitic rats, and based upon this study fluvoxamine has been suggested as the anti-depressant medication of choice in IBD (Minaiyan, Hajhashemi et al. 2015). This is not currently clinical practice.

Table 4.57.9: Mass spectra search for 194.9948803 m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB60500	NTP	M+2H	194.993551	387.97255	0.0013293	c-glycosyl compounds
HMDB06284	L-2,4-diaminobutyric acid	M+2K+H	194.993268	118.074228	0.0016123	Carboxylic acids and derivatives
HMDB02362	2,4-Diaminobut	M+2K+H	194.993268	118.074228	0.0016123	Carboxylic acids and

	yric acid					derivatives
HMDB59899	alpha-Methylstyrene	M+2K+H	194.99729	118.07825	0.0024097	Benzene and substituted derivatives
HMDB59837	Indane	M+2K+H	194.99729	118.07825	0.0024097	Indanes
HMDB02151	Dimethyl sulfoxide	2M+K	194.991029	78.013936	0.0038513	Sulfoxides
HMDB40363	(±)-Glycerol 1-monophosphate Mg salt (1:1)	M+H	194.990342	193.983066	0.0045383	Not classified
HMDB37851	Apigenin 7-sulfate	M+H+K	194.990036	350.009638	0.0048443	Flavonoids
HMDB61204	Difluorodeoxyuridine monophosphate	M+2Na	195.000272	344.022108	0.0053917	Pyrimidine nucleosides
HMDB38471	Luteolin 4'-sulfate	M+H+Na	195.000523	366.004553	0.0056427	Flavonoids

Figure 4.109: Boxplot Urinary Variable ID 373 Treatment Naïve v Healthy Controls UHPLC-FTMS



p=0.00015633

In this experiment, peak intensities of urinary variable ID 373 are lower in treatment naïve IBD patients than in healthy controls. Nucleoside triphosphate and indane are identified as metabolites of

interest.

Nucleoside Triphosphate

Nucleoside triphosphate (NTP) belongs to the class of organic compounds known as c-glycosyl compounds. NTPs generally provide energy and a phosphate group for phosphorylations. Adenosine triphosphate is an example of a natural nucleoside triphosphate that is an essential activator of inflammasomes (Cauwels, Rogge et al. 2014). Anti-inflammatory medications such as salicylates, methotrexate and purine analogs like 6-MP and cyclosporine, exert their therapeutic actions in inflammatory diseases by decreasing intracellular adenosine 5'-triphosphate concentrations and increasing extracellular adenosine levels. Adenosine contributes to the resolution of inflammation, both by down-regulating macrophage activation and by advancing Th2- *vs* Th1-cell response (Ye, Rajendran 2009). In our study we see lower levels of NTP (potentially ATP) in the urine of treatment naïve patients. This may represent little being secreted as it is being utilised in the body as a proinflammatory molecule.

Indane

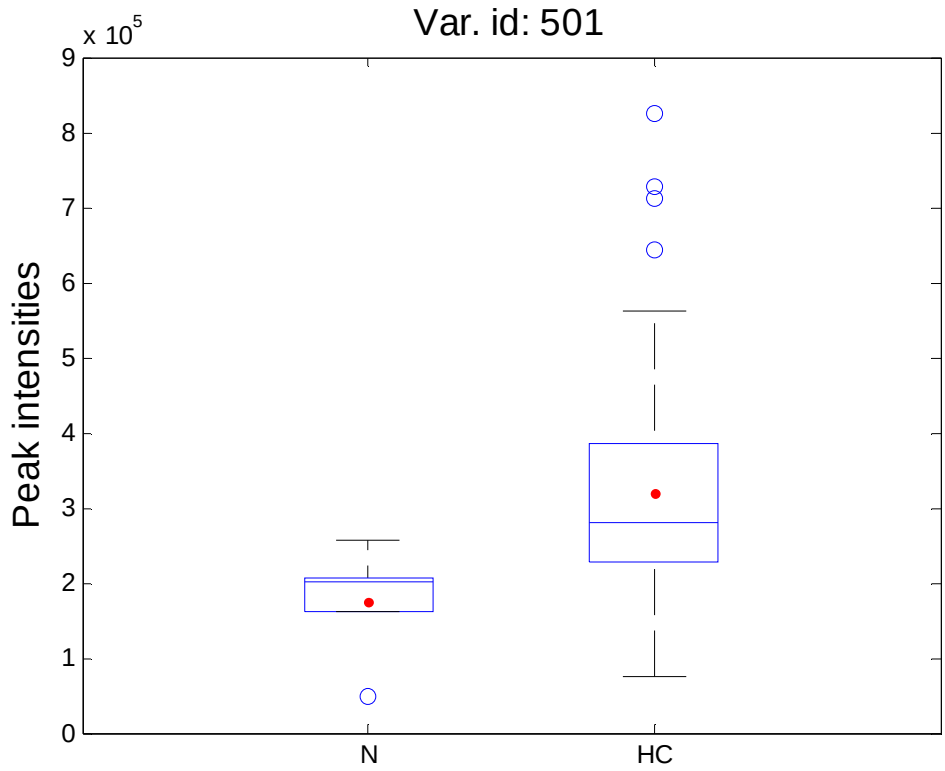
Indane, a hydrocarbon petrochemical compound, belongs to the class of organic compounds known as indanes. The indane skeleton is found in therapeutic molecules in medicinal chemistry. A novel indane, PH46A, optimised for anti-inflammatory and bioavailability, has been shown to reduce reduce histological damage in murine colitis models (Frankish, Sheridan 2012). This compound may have potential as a treatment in the future.

Table 4.57.10: Mass spectra search for 224.0294257 m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB00992	3-Succinoylpyridine	M+2Na-H	224.029403	179.058243	0.0000227	Keto acids and derivatives
HMDB00714	Hippuric acid	M+2Na-H	224.029403	179.058243	0.0000227	Benzene and substituted derivatives
HMDB12884	Adrenochrome	M+2Na-H	224.029403	179.058243	0.0000227	Not classified
HMDB32398	Methyl n-formylanthranilate	M+2Na-H	224.029403	179.058243	0.0000227	Benzene and substituted derivatives
HMDB32595	1-(4-Methoxyphenyl)-2-nitroethylene	M+2Na-H	224.029403	179.058243	0.0000227	Benzene and substituted derivatives
HMDB01117	4-Phosphopantothenoylcy	M+2Na	224.032312	402.086188	0.0028863	Carboxylic acids and derivatives

	steine					
HMDB15244	Cefuroxime	M+H+Na	224.032689	424.068884	0.0032633	Lactams
HMDB29442	(R)C(R)S-S-Propylcysteine sulfoxide	M+2Na-H	224.032774	179.061614	0.0033483	Carboxylic acids and derivatives
HMDB31340	Cyclamic acid	M+2Na-H	224.032774	179.061614	0.0033483	Sulfamic acid derivatives
HMDB14868	Thiabendazole	M+Na	224.025286	201.036068	0.0041397	Benzimidazoles

Figure 4.110: Boxplot Urinary Variable ID 501 Treatment Naïve v Healthy Controls UHPLC-FTMS



p=4.60E-05

In this experiment, lower peak intensities of urinary variable 501 are seen in treatment naïve IBD patients than in HCs. Hippuric acid and adenochrome are identified as metabolites of interest.

Hippuric Acid

Hippuric acid is an acyl glycine formed by the conjugation of benzoic acid with glycine. It is a gut-bacteria regulated metabolite that has been show to be reduced in IBD. This is likely due to alteration in the gut microbiota rather than dietary benzoate intake, although the specific gut flora remain unidentified (Williams, Cox et al. 2010). This finding is replicated in our study.

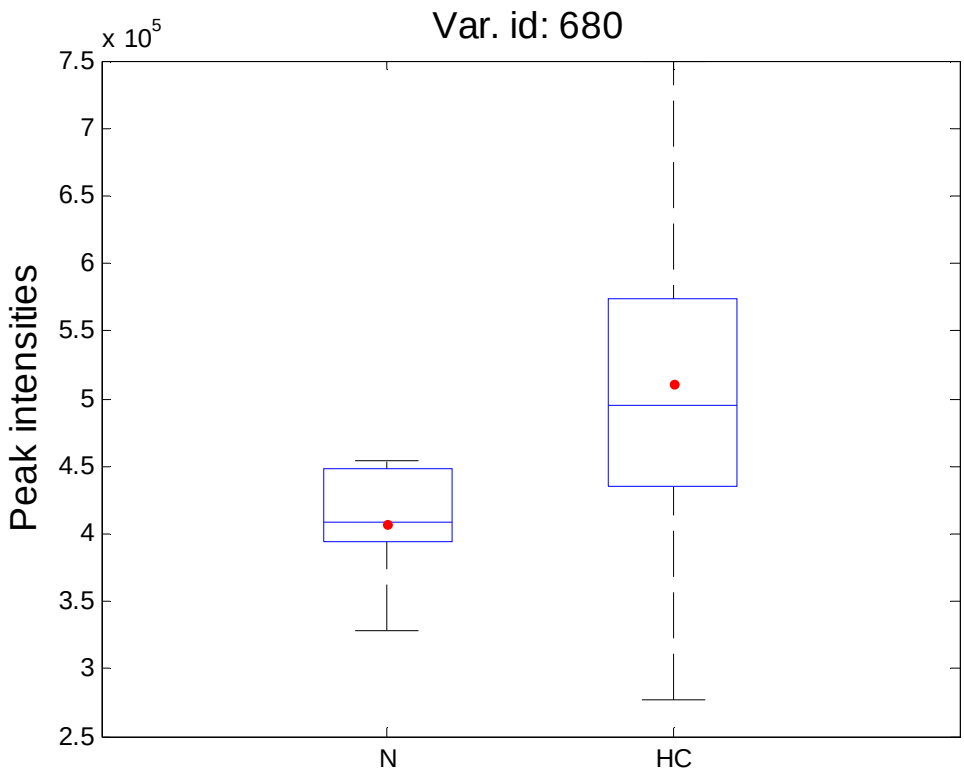
Adrenochrome

Adrenochrome is a pigment obtained by the oxidation of adrenaline. This metabolite was identified as being less abundant in CD patients than in HCs in a previous study (Johnston 2014), and although the reason remains unclear, we have duplicated these results in our study.

Table 4.57.11: Mass spectra search for 279.1330075 m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB36390	3-Phenylpropyl hexanoate	M+2Na-H	279.13314	234.16198	0.0001325	Fatty acyls
HMDB36466	Zerumbone oxide	M+2Na-H	279.13314	234.16198	0.0001325	Carbonyl compounds
HMDB36035	Marasmene	M+2Na-H	279.13314	234.16198	0.0001325	Furofurans
HMDB38795	7,9-Illudadiene-3,14-diol	M+2Na-H	279.13314	234.16198	0.0001325	Not classified
HMDB32081	4,10-Longipinanedione	M+2Na-H	279.13314	234.16198	0.0001325	Prenol lipids
HMDB35202	7-Hydroxycostal	M+2Na-H	279.13314	234.16198	0.0001325	Not classified
HMDB35889	Germacrone 4,5-epoxide	M+2Na-H	279.13314	234.16198	0.0001325	Prenol lipids
HMDB41036	Sugeonol	M+2Na-H	279.13314	234.16198	0.0001325	Prenol lipids
HMDB37710	4-Methylphenyl octanoate	M+2Na-H	279.13314	234.16198	0.0001325	Benzene and substituted derivatives
HMDB38190	Rishitinol	M+2Na-H	279.13314	234.16198	0.0001325	Not classified

Figure 4.111: Boxplot Urinary Variable ID 680 Treatment Naïve v Healthy Controls UHPLC-FTMS



p=5.31E-05

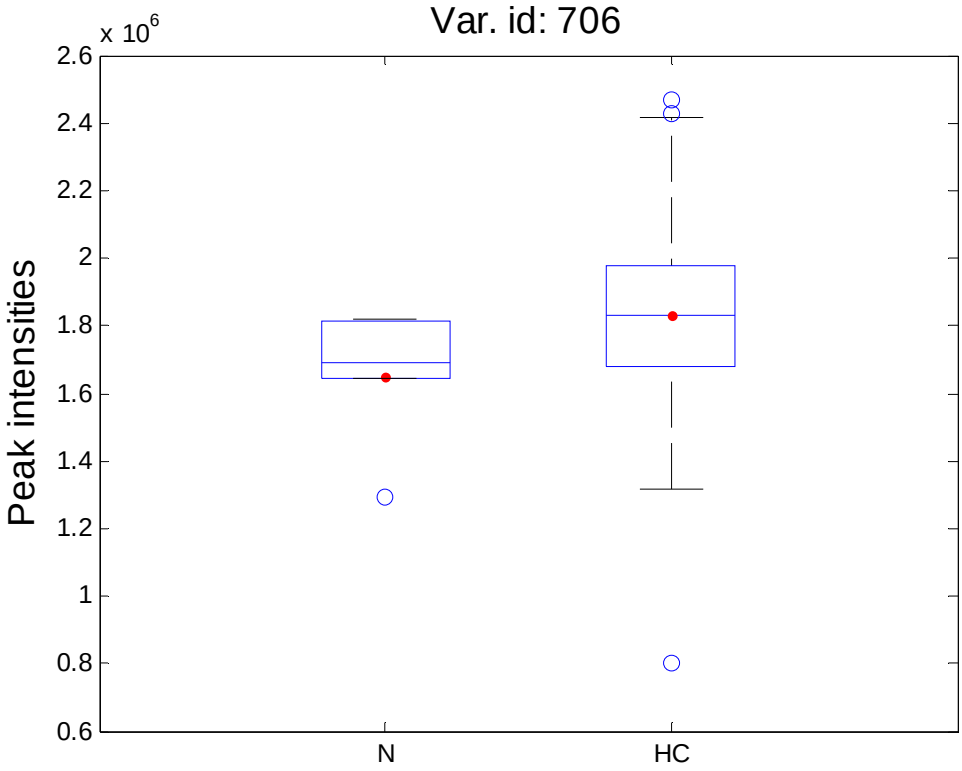
In this experiment, peak intensities of urinary variable ID 680 are lower in treatment naïve IBD patients than in healthy controls. All of the metabolites identified in this group are dietary in nature.

Table 4.57.12: Mass spectra search for 287.099141 m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB40647	16,17-Dihydro-16a,17-dihydroxygibberellin A4 17-glucoside	M+2Na	287.099556	528.220677	0.000415	Prenol lipids
HMDB15258	Dutasteride	M+2Na	287.099792	528.221147	0.000651	Steroids and steroid derivatives
HMDB61136	di-Hydroxymelatonin	M+Na	287.100225	264.111007	0.001084	Indoles and derivatives
HMDB04259	Acetyl-N-formyl-5-methoxykynurenamine	M+Na	287.100225	264.111007	0.001084	Benzene and substituted derivatives
HMDB063	Alpha-N-	M+Na	287.100225	264.111007	0.001084	Carboxylic

44	Phenylacetyl-L-glutamine					acids and derivatives
HMDB61182	Dihydro-5-Hydroxyrofecoxib	M+2Na+H	287.100684	332.071844	0.001543	Dihydrothiophines
HMDB30557	Trachelosside	M+H+Na	287.10076	550.205027	0.001619	Not classified
HMDB38718	Scorzonoside	M+H+Na	287.10076	550.205027	0.001619	2-arylbenzofuran flavonoids
HMDB14831	Fluorescein	M+2Na+H	287.097313	332.068473	0.001828	Benzopyrans
HMDB38230	Bn-NCC-2	M+3Na	287.096481	792.321788	0.00266	Tetrapyrroles and derivatives

Figure 4.112: Boxplot Urinary Variable ID 706 Treatment Naïve v Healthy Controls UHPLC-FTMS



p=0.00019558

In this experiment, reduced peak intensities of urinary variable ID 706 are seen in treatment naïve IBD patients compared to healthy controls. Di-Hydroxymelatonin, Acetyl-N-formyl-5-methoxykynurenamine, and Tracheloside are metabolites of biological interest.

Di-Hydroxymelatonin

Di-Hydroxymelatonin, a metabolite of melatonin, belongs to the class of organic compounds known as serotonins. Melatonin is a powerful antioxidant and free radical scavenger. It also decreases levels of TNF-α and can inhibit NF-κB. Melatonin can prevent DSS-induced coltis in mice and rat models. In these models, melatonin reversed both the increase in intestinal permeability and influx of bacterial endotoxins, and decreased myeloperoxidase and TNF-α activity (Swanson, Burgess et al. 2011). In our study, lower levels of a melatonin metabolite are seen in treatment naïve IBD patients than in healthy controls.

Acetyl-N-formyl-5-methoxykynurenamine

Acetyl-N-formyl-5-methoxykynurenamine, belonging to the class of organic compounds known as phenylpropylamines, results from the oxidative cleavage of the pyrrole ring during melatonin oxidation by myeloperoxidase, a superoxide anion (O)-dependent reaction. Melatonin is discussed above.

Tracheloside

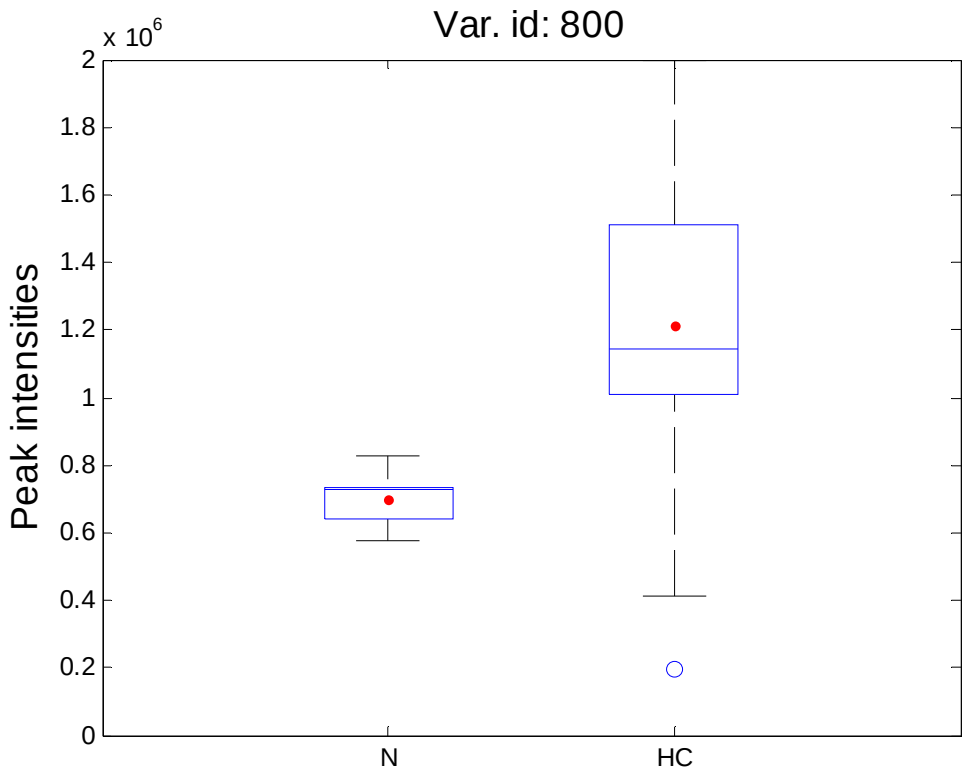
Tracheloside, found in fats and oils, belongs to the family of lignan glycosides. Trachelogenin, an aglycone of tracheloside (Shin, Bae et al. 2015), has been shown to enhance intestinal barrier function, and is seen in greater levels in HCs than in IBD in our study.

Table 4.57.13: Mass spectra search for 334.0909909 m/z

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta	Class
HMDB30704	Taxiphyllin	M+Na	334.08972	311.100502	0.0012709	Carbohydrates and carbohydrate conjugates
HMDB60471	Dhurrin	M+Na	334.08972	311.100502	0.0012709	Carbohydrates and carbohydrate conjugates
HMDB37841	N-(1-Deoxy-1-fructosyl)methionine	M+Na	334.093091	311.103873	0.0021001	Carbohydrates and carbohydrate conjugates
HMDB15585	Chlophedianol	M+2Na-H	334.094502	289.123342	0.0035111	Benzene and substituted derivatives
HMDB60463	Citalopram propionic acid	M+Na	334.08499	311.095772	0.0060009	Not classified
HMDB14045	3-Methoxymo	M+2K+H	334.097004	257.177964	0.0060131	Morphinans

	rphinan					
HMDB60552	Dextrorphan	M+2K+H	334.097004	257.177964	0.0060131	Morphinans
HMDB14992	Levorphanol	M+2K+H	334.097004	257.177964	0.0060131	Morphinans
HMDB39087	Sudachiin B	M+2H	334.097076	666.1796	0.0060851	Flavanoids
HMDB39088	Sudachiin C	M+2H	334.097076	666.1796	0.0060851	Not classified

Figure 4.111: Boxplot Urinary Variable ID 800 Treatment Naïve v Healthy Controls UHPLC-FTMS



p=1.08E-05

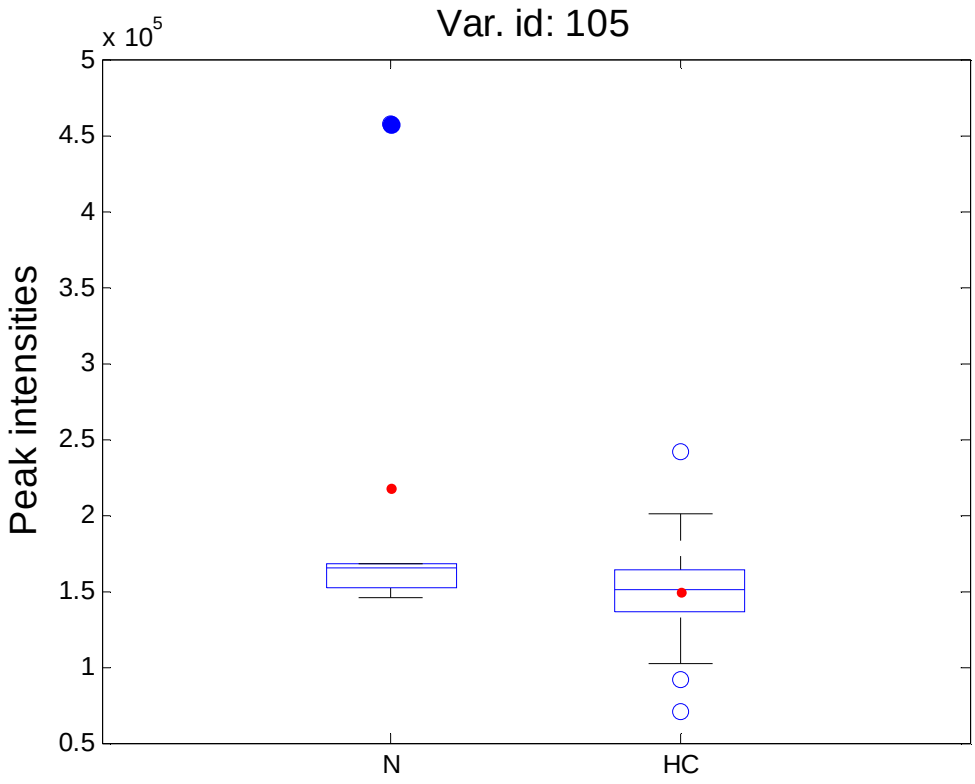
In this experiment increased peak intensities of urinary variable ID 800 are seen in healthy controls compared to treatment naïve IBD patients. This group of metabolites have been discussed previously, and the finding are likely related to dietary intake.

4.18.2 Experiment 16.2: Metabolite Identification Treatment Naïve v Healthy Controls GC-ToF-MS

Table 4.58: Putative Metabolites Treatment Naïve v Healthy Controls GC-ToF-MS

Variable ID	Biofluid	Quant mass	Retention time	Putative match	p value	q value
105	Urine	91	1222.228	Hydroxylamine	0.0020794	0.0374292
186	Urine	143	1024.129	Cellobiose	0.00046286	0.00833148
338	Urine	259	1024.928	Cellobiose	0.0018524	0.0333432

Figure 4.114: Boxplot Urinary Variable ID 105 Hydroxylamine Treatment Naïve v Healthy Controls GC-ToF-MS



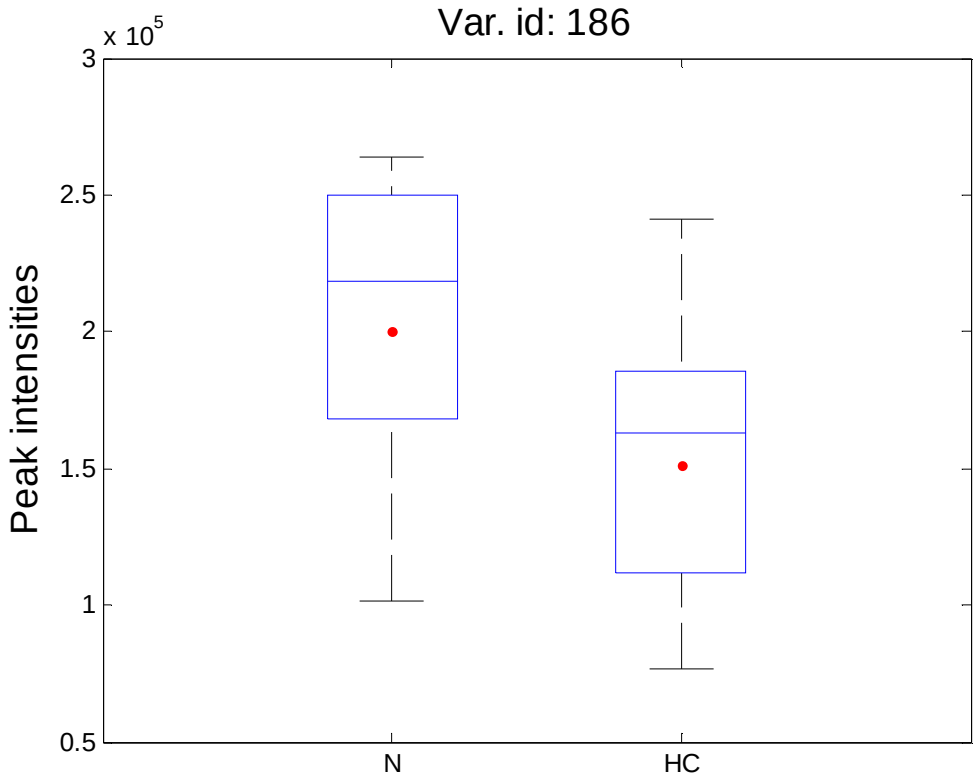
p=0.0020794

In this experiment, increased peak intensities of hydroxylamine are seen in the urine of treatment naïve IBD patients compared to HCs.

Hydroxylamine

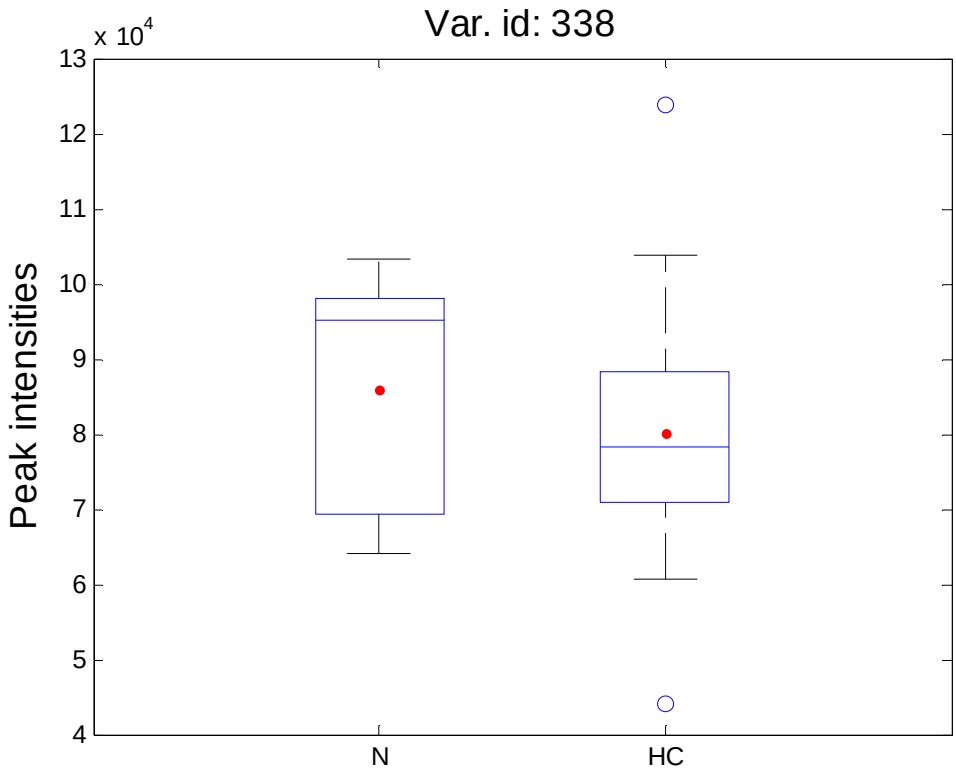
Hydroxylamine, utilised in the preparation of oximes and as an intermediate in biological nitrification, belongs to the class of inorganic compounds known as homogeneous other non-metal compounds. No pathogonomic links are to IBD are identified and these findings are unexplained.

Figure 4.115: Boxplot Urinary Variable ID 186 Cellobiose Treatment Naïve v Healthy Controls GC-ToF-MS



p=0.00046286

Figure 4.116: Boxplot Urinary Variable ID 338 Cellobiose Treatment Naïve v Healthy Controls GC-ToF-MS



p=0.0018524

In our study we see higher peak intensities of cellobiose in the urine of treatment naïve patients than in HCs. Cellobiose has been previously discussed. In this treatment naïve IBD patient group, this may indicate the break down cellulose into cellobiose to attempt to utilise its protective effects.

4.18.3 Experiment 16 Summary

Serum metabolites in the class benzenes and substituted derivatives, and urinary metabolites in the class carbohydrates and carbohydrate conjugates, indoles and derivatives, and benzene and derivatives are reduced in drug naïve IBD patients compared to healthy controls. Metabolites in the class homogenous other non-metal compounds are increased in the urine of treatment naïve IBD patients compared to healthy controls.

4.19 Crohn's Disease Behaviour, Location, Age at Diagnosis and HBI

The following experiments, based upon the longitudinal group, aim to determine whether the variables identified in the Montreal Classification, and the disease activity score (HBI), have discernable metabolomic profiles.

Table 4.59 Experiment 17-20 number of samples analysed

	Crohn's Disease
Serum samples	128
Urine samples	127

4.19.1 Experiment 17: Crohn's Disease Behaviour Differentiation

In experiment 17 we consider the group of patients with Crohn's disease, and aim to determine whether disease behaviour can be differentiated by the metabolic profile. The variables have been collected as part of the Montreal Classification;

B1 non-stricturing non-penetrating,

B2 stricturing,

B3 penetrating,

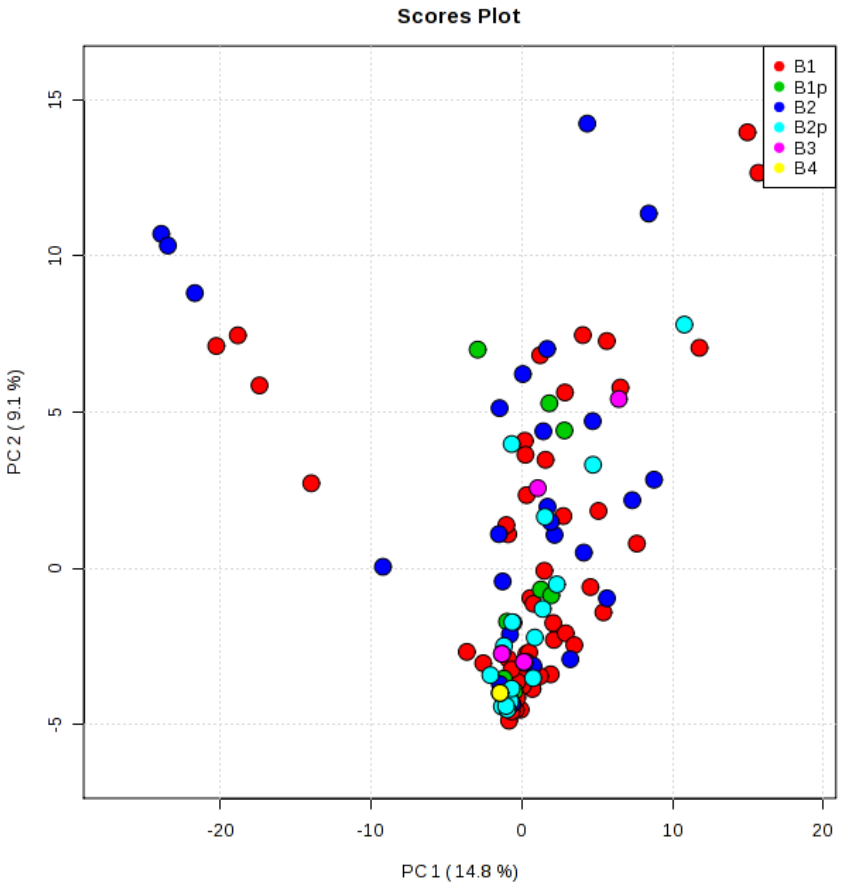
p concomintant perianal disease.

The label B4 was allocated to a patient with stricturing and penetrating disease.

Results are presented by means of principle component analysis plots. PCA allows us to find patterns in data of high dimensions, and expressing the data in such a way to highlight the similarities and differences. The advantage of using PCA is that once patterns are identified, data is compressed by reducing the number of dimensions, without much loss of information. When plotting PCA, each axis has an eigenvalue associated with it. The eigenvalue is the amount of variation explained by the axis, and is typically expressed as a percentage of the total.

4.19.2 Experiment 17.1: Crohn's Disease Behaviour Differentiation Urine Analysis UHPLC-FTMS

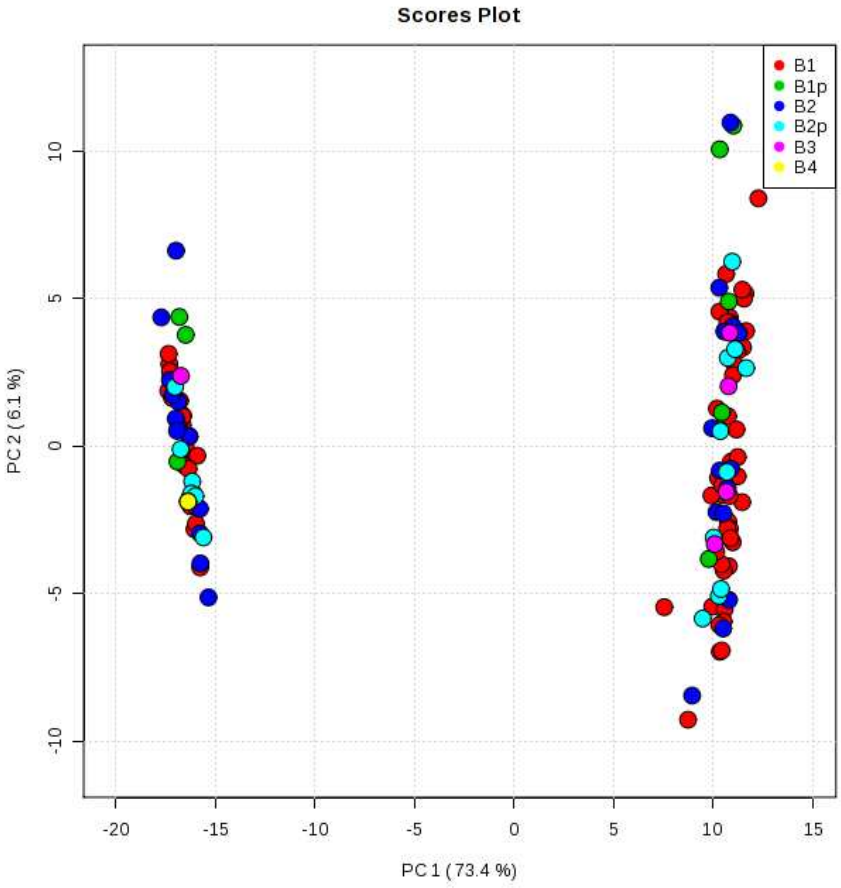
Figure 4.117: PCA Plot UHPLC-FTMS Urine Analysis Grouped by Disease Behaviour



No differentiation is seen between the metabolomic profiles of urine samples from patients with Crohn's Disease in relation to disease behaviour on the UHPLC-FTMS platform.

4.19.3 Experiment 17.2: Crohn's Disease Behaviour Differentiation Urine Analysis GC-ToF-MS

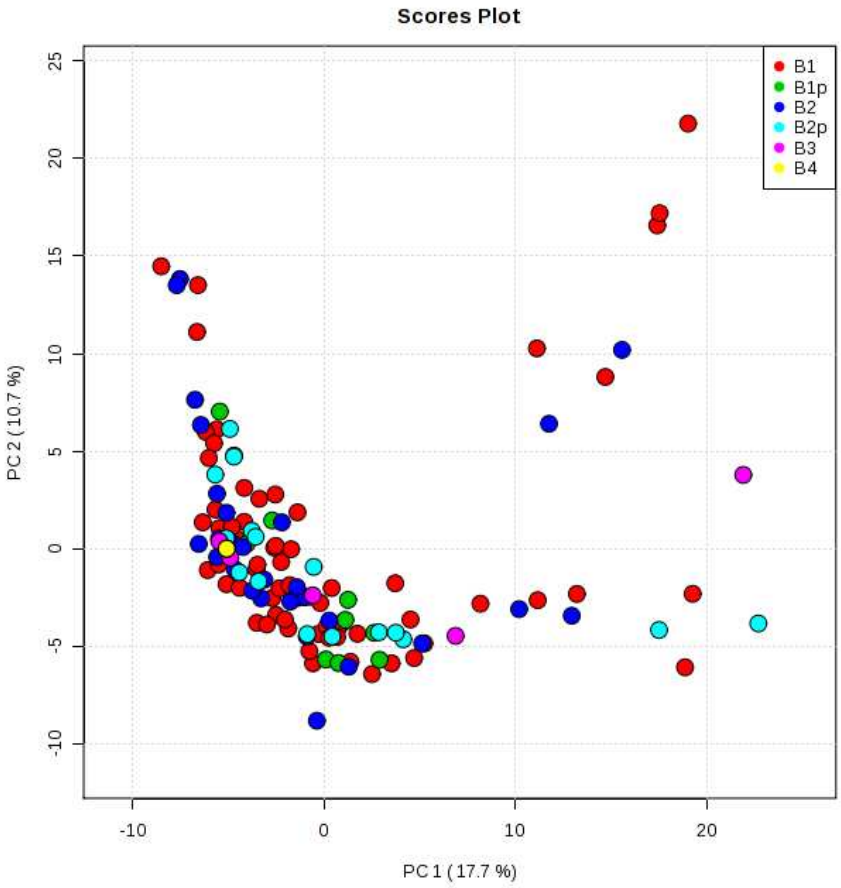
Figure 4.118: PCA Plot GC-ToF-MS Urine Analysis Grouped by Disease Behaviour



No differentiation is seen between the metabolomic profiles of urine samples from patients with Crohn's Disease in relation to disease behaviour on the GC-ToF-MS platform.

4.19.4 Experiment 17.3: Crohn's Disease Behaviour Differentiation Serum Analysis UHPLC-FTMS

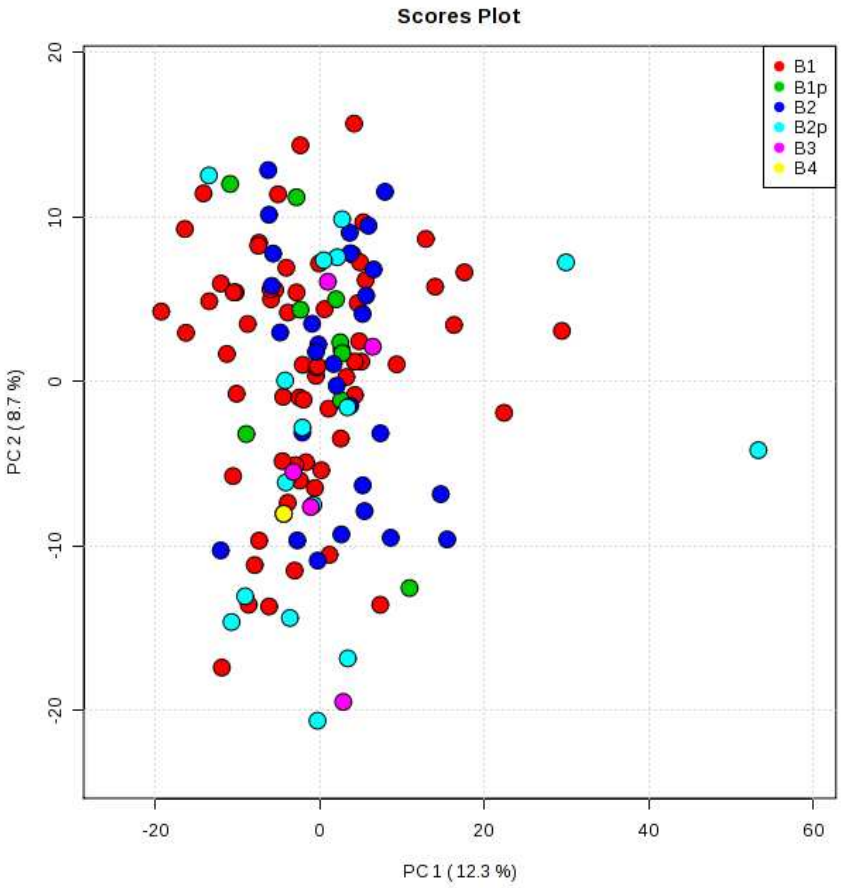
Figure 4.119: PCA Plot UHPLC-FTMS Serum Analysis Grouped by Disease Behaviour



No differentiation is seen between the metabolomic profiles of serum samples from patients with Crohn's Disease in relation to disease behaviour on the UHPLC-FTMS platform.

4.19.5 Experiment 17.4: Crohn's Disease Behaviour Differentiation Serum Analysis GC-ToF-MS

Figure 4.120: PCA Plot GC-ToF-MS Serum Analysis Grouped by Disease Behaviour



No differentiation is seen between the metabolomic profiles of serum samples from patients with Crohn's Disease in relation to disease behaviour on the GC-ToF-MS platform.

4.19.6 Experiment 17 Summary

In our study we have not been able to show differentiation between the metabolomic profiles of Crohn's Disease patients in relation to the disease behaviour they are displaying, using either serum or urine samples, on UHPLC-FTMS or GC-ToF-MS platforms.

4.20 Experiment 18: Crohn's Disease Location Differentiation

In experiment 18 we consider the group of patients with Crohn's disease, and aim to determine whether disease location can be differentiated by the metabolic profile. The variables have been collected as part of the Montreal Classification;

L1 ileal,

L2 colonic,

L3 ileocolonic,

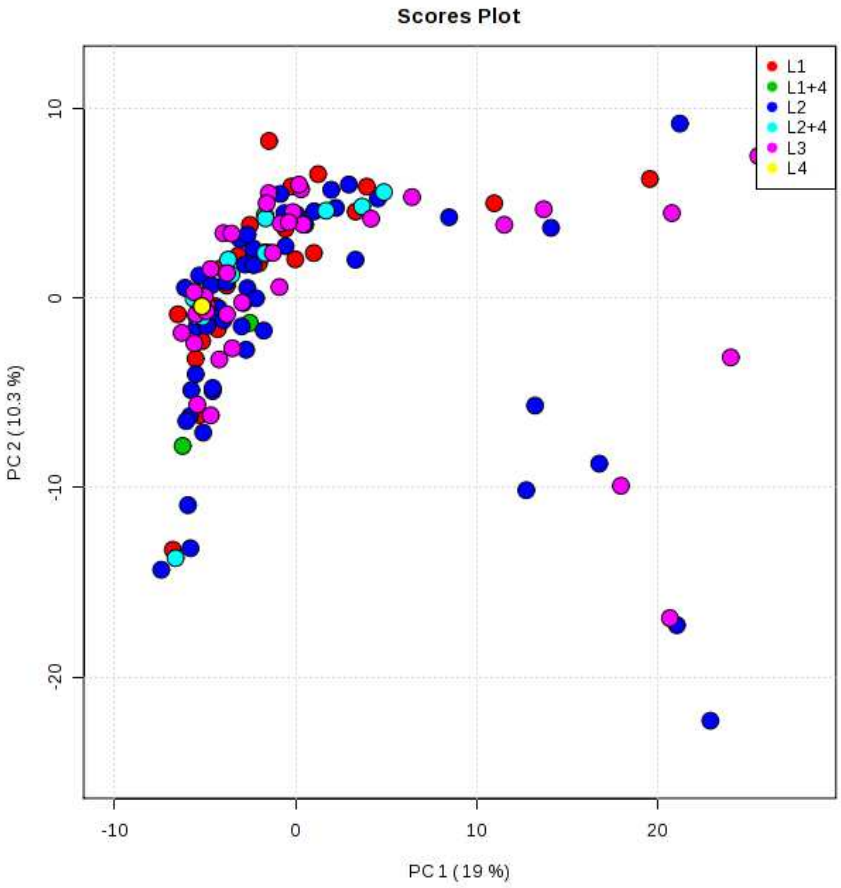
L4 isolated upper GI diease*.

*L4 is a modified that can be added to L1–L3 when concominant upper GI disease is present.

As in experiment 17, the results are presented by means of PCA plots.

4.20.1 Experiment 18.1: Crohn's Disease Location Differentiation Urine Analysis UHPLC-FTMS

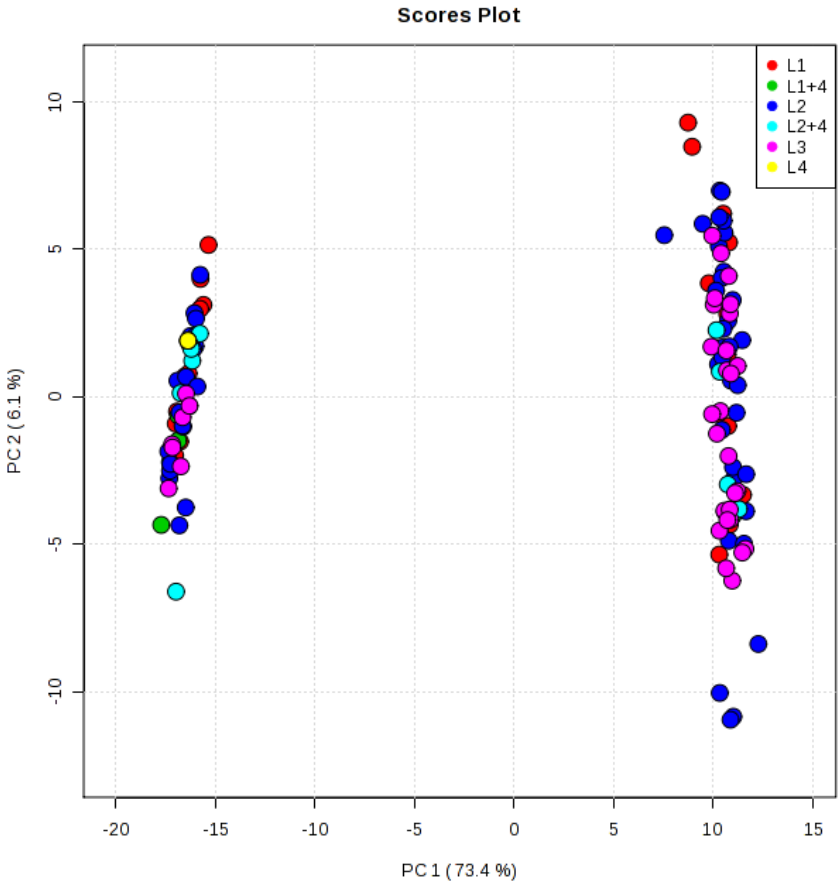
Figure 4.121: PCA Plot UHPLC-FTMS Urine Analysis Grouped by Disease Location



No differentiation is seen between the metabolomic profiles of urine samples from patients with Crohn's Disease in relation to disease location on the UHPLC-FTMS platform.

4.20.2 Experiment 18.2: Crohn's Disease Location Differentiation Urine Analysis GC-ToF-MS

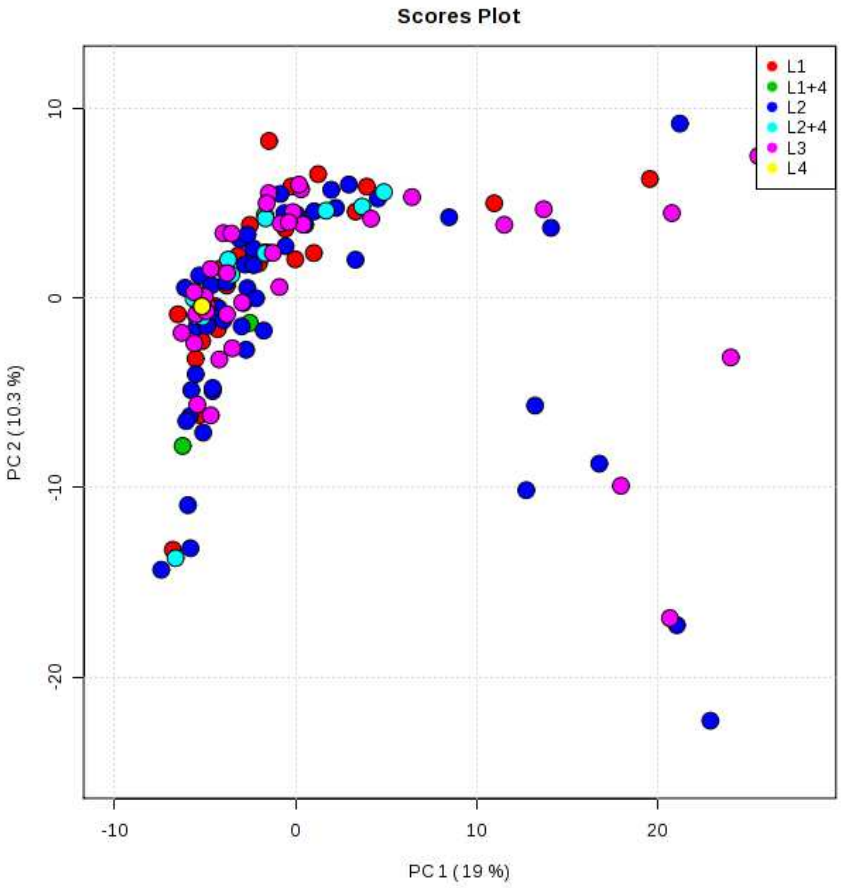
Figure 4.122: PCA Plot GC-ToF-MS Urine Analysis Grouped by Disease Location



No differentiation is seen between the metabolomic profiles of urine samples from patients with Crohn's Disease in relation to disease location on the GC-ToF-MS platform.

4.20.3 Experiment 18.3: Crohn's Disease Location Differentiation Serum Analysis UHPLC-FTMS

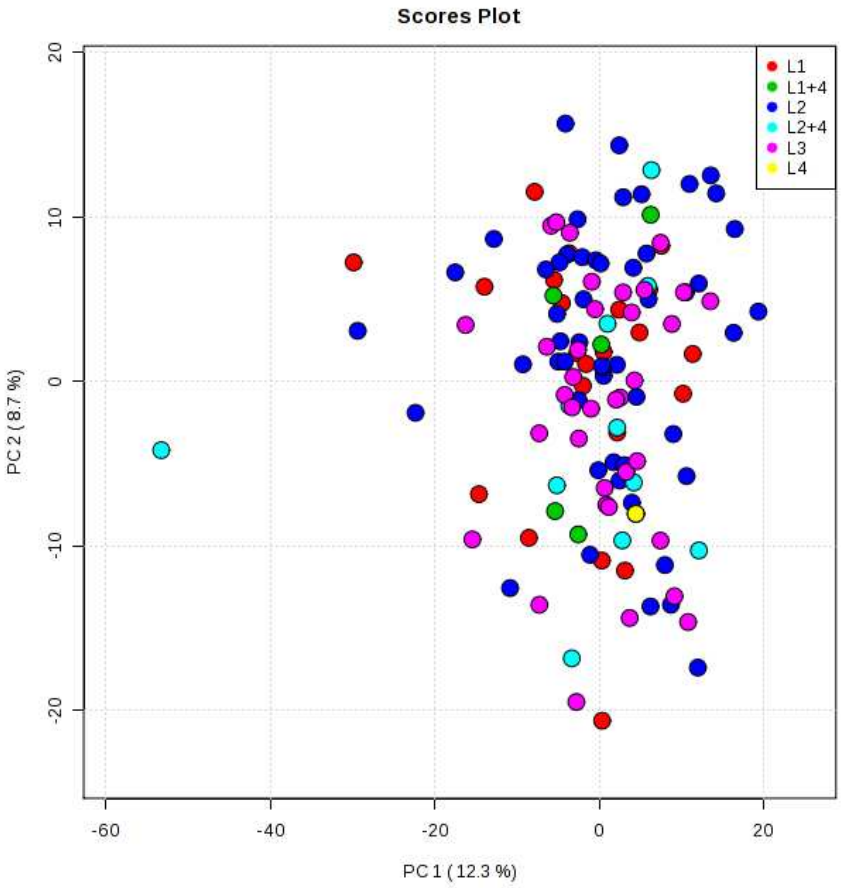
Figure 4.123: PCA Plot UHPLC-FTMS Serum Analysis Grouped by Disease Location



No differentiation is seen between the metabolomic profiles of serum samples from patients with Crohn's Disease in relation to disease location on the UHPLC-FTMS platform.

4.20.4 Experiment 18.4: Crohn's Disease Location Differentiation Serum Analysis GC-ToF-MS

Figure 4.124: PCA Plot GC-ToF-MS Serum Analysis Grouped by Disease Location



No differentiation is seen between the metabolomic profiles of serum samples from patients with Crohn's Disease in relation to disease location on the GC-ToF-MS platform.

4.20.5 Experiment 18 Summary

In our study we have not been able to show differentiation between the metabolomic profiles of Crohn's Disease patients in relation to the disease location, using either serum or urine samples, on UHPLC-FTMS or GC-ToF-MS platforms.

4.21 Experiment 19: Crohn's Disease Age at Diagnosis Differentiation

In experiment 19 we consider the group of patients with Crohn's disease, and aim to determine whether age at diagnosis can be differentiated by the metabolic profile. The variables have been collected as part of the Montreal Classification;

A1 below 16 years,

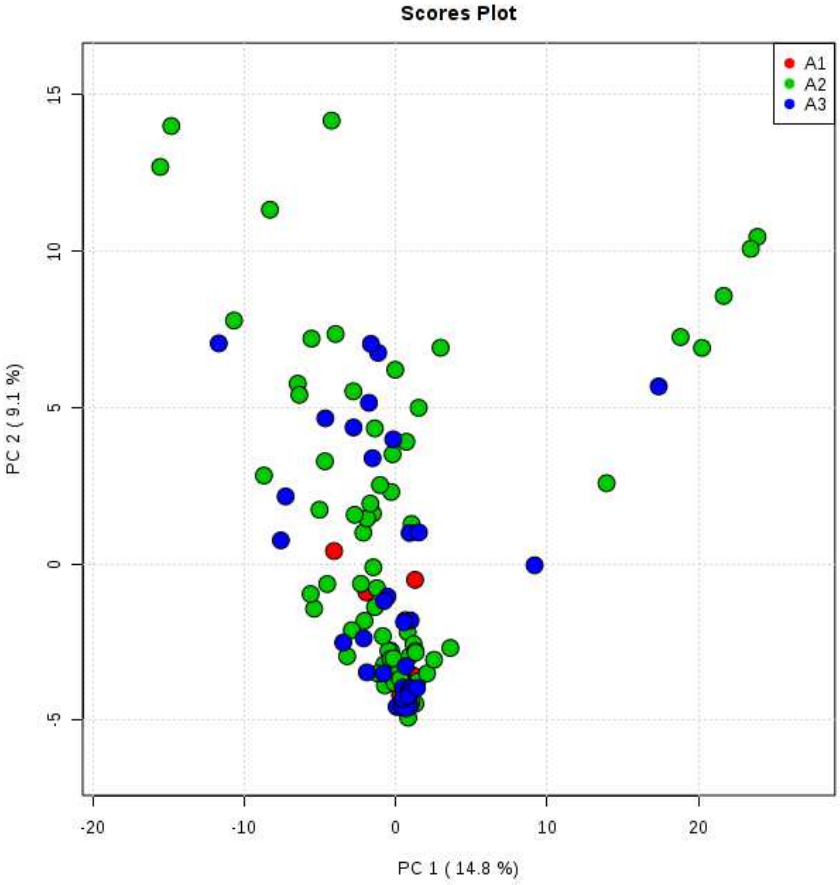
A2 between 17 and 40 years,

A3 above 40 years,

As in experiment 17 and 18, the results are presented by means of PCA plots.

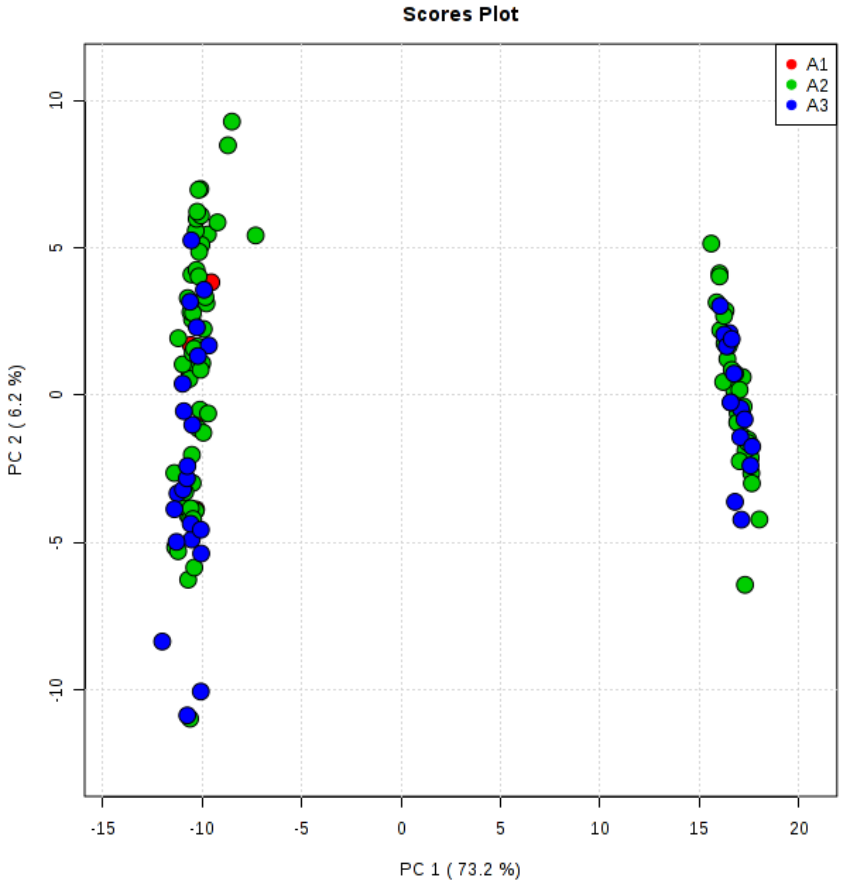
4.21.1 Experiment 19.1: Crohn's Disease Age at Diagnosis Differentiation Urine Analysis UHPLC-FTMS

Figure 4.125: PCA Plot UHPLC-FTMS Urine Analysis Grouped by Age at Diagnosis



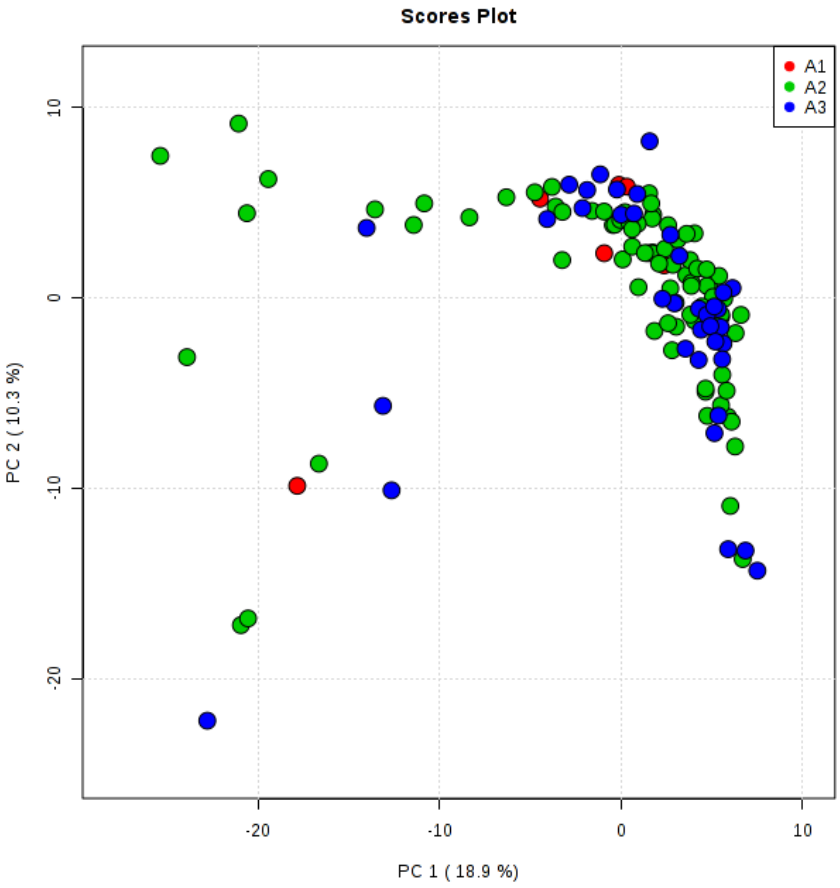
4.21.2 Experiment 19.2: Crohn's Disease Age at Diagnosis Differentiation Urine Analysis GC-ToF-MS

Figure 4.126: PCA Plot GC-ToF-MS Urine Analysis Grouped by Age at Diagnosis



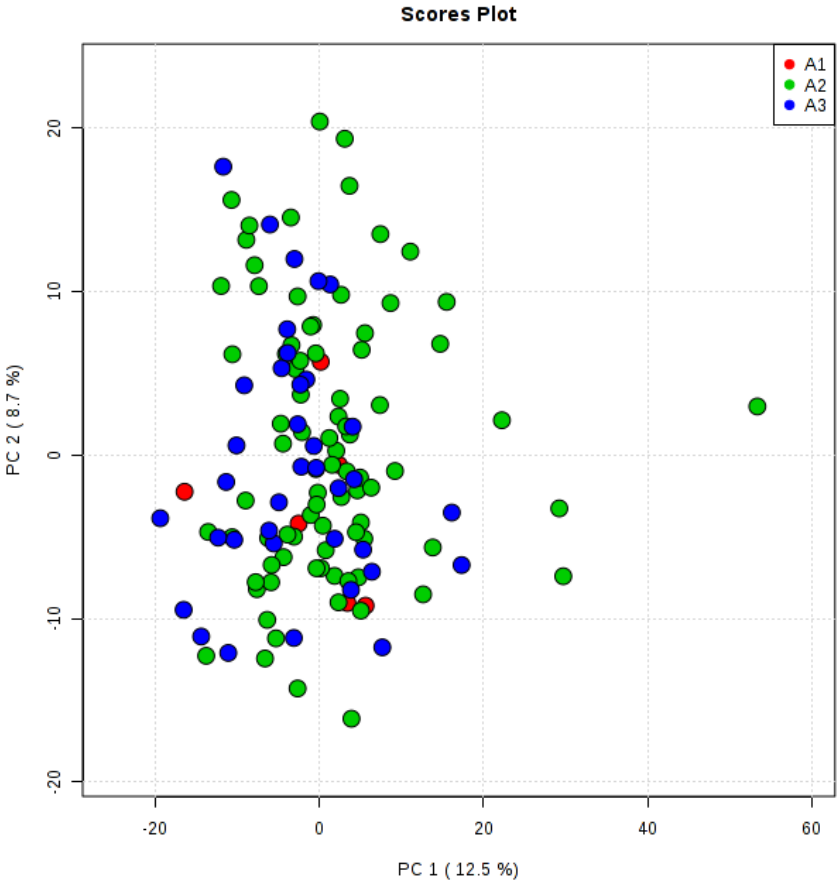
4.21.3 Experiment 19.3: Crohn's Disease Age at Diagnosis Differentiation Serum Analysis UHPLC-FTMS

Figure 4.127: PCA Plot UHPLC-FTMS Serum Analysis Grouped by Age at Diagnosis



4.21.4 Experiment 19.4: Crohn's Disease Age at Diagnosis Differentiation Serum Analysis GC-ToF-MS

Figure 4.128: PCA Plot GC-ToF-MS Serum Analysis Grouped by Age at Diagnosis



4.21.5 Experiment 19 Summary

In our study we have not been able to show differentiation between the metabolomic profiles of Crohn's Disease patients in relation to the age at disease diagnosis, using either serum or urine samples, on UHPLC-FTMS or GC-ToF-MS platforms.

4.22 Experiment 20: Crohn's Disease Differentiation by HBI

In Experiment 20 we consider the group of patients with Crohn's disease, and aim to determine whether the disease activity score (HBI) can be differentiated by the metabolic profile.

Spearman's rank correlation coefficient (r_s) is a nonparametric measure of statistical dependence between two variables. The sign of the Spearman correlation indicates the direction of association between x (the independent variable) and y (the dependent variable). If y tends to increase when x increases, the Spearman correlation coefficient is positive. If y tends to decrease when x increases, it is negative. When x and y are perfectly monotonically related, the Spearman correlation coefficient becomes +1 or -1. Correlation is an effect size, thus we can describe the strength of the correlation.

Table 4.60: Strength of the Spearman's rank correlation coefficient

r_s	Strength of the correlation
0.00 – 0.19	Very Weak
0.20 – 0.39	Weak
0.40 – 0.59	Moderate
0.60 – 0.79	Strong
0.80 – 1.0	Very Strong

As previously discussed, due to the complexity of the data analysed in the longitudinal group with multiple testing, FDR correction rather than Bonferroni is used in the following experiments.
In this experiment, variables were deemed significant if both the p and the q value were <0.05.

4.22.1 Experiment 20.1: Crohn's Disease Differentiation by HBI Urine Analysis UHPLC-FTMS

Table 4.61: Important Variables Identified UHPLC-FTMS Urine Analysis by HBI

Variable ID	Spearman Rank	p value	q value
646	0.2443	0.0063	0.946268605
667	-0.2339	0.0089	0.946268605
60	0.216	0.016	0.946268605
446	-0.197	0.0283	0.946268605
111	0.1965	0.0287	0.946268605
471	0.1954	0.0297	0.946268605
617	0.1915	0.0331	0.946268605
180	0.1912	0.0334	0.946268605
194	0.1895	0.035	0.946268605
566	0.1856	0.039	0.946268605
622	0.1844	0.0403	0.946268605
407	-0.1788	0.0469	0.946268605
371	0.1785	0.0473	0.946268605

All of the variables identified were very weakly or weakly associated with the HBI. None of the variables identified maintained significance in relation to the HBI following FDR correction (q value).

4.22.2 Experiment 20.2: Crohn's Disease Differentiation by HBI Urine Analysis GC-ToF-MS

Table 4.62: Important Variables Identified GC-ToF-MS Urine Analysis by HBI

Variable ID	Spearman Rank	p value	q value
208	0.2178	0.0143	0.926296678
44	0.2085	0.0192	0.926296678
29	0.1826	0.0407	0.926296678
24	0.1802	0.0435	0.926296678

All of the variables identified were very weakly or weakly associated with the HBI. None of the variables identified maintain significance in relation to the HBI following FDR correction (q value).

4.22.3 Experiment 20.3: Crohn's Disease Differentiation by HBI Serum Analysis UHPLC-FTMS

Table 4.63: Important Variables Identified UHPLC-FTMS Serum Analysis by HBI

Variable ID	Spearman Rank	p value	q value
178	0.2535	0.004	0.353871429
23	-0.2514	0.0044	0.353871429
443	0.2483	0.0049	0.353871429
105	0.2423	0.0061	0.353871429
318	-0.2412	0.0063	0.353871429
396	-0.2414	0.0063	0.353871429
436	-0.2384	0.0069	0.353871429
163	0.2259	0.0107	0.381845455
174	0.2255	0.0108	0.381845455
496	-0.2256	0.0108	0.381845455
55	-0.2232	0.0117	0.381845455
442	0.2143	0.0155	0.463708333
399	-0.21	0.0178	0.470923529
9	-0.2077	0.0191	0.470923529
16	-0.2033	0.0219	0.470923529
27	0.2027	0.0223	0.470923529
341	-0.2027	0.0223	0.470923529
235	-0.1957	0.0274	0.521494737
226	-0.1955	0.0276	0.521494737
15	-0.1906	0.0318	0.547047619
354	-0.1904	0.032	0.547047619
548	-0.1877	0.0346	0.564609091
195	-0.1855	0.0368	0.567872727
30	0.1843	0.0381	0.567872727
462	-0.1819	0.0407	0.567872727
299	-0.1768	0.0468	0.567872727
482	-0.1755	0.0484	0.567872727

All of the variables identified were very weakly or weakly associated with the HBI. None of the variables identified maintain significance in relation to the HBI following FDR correction (q value).

4.22.4 Experiment 20.4: Crohn's Disease Differentiation by HBI Serum Analysis GC-ToF-MS

Table 4.64: Important Variables Identified GC-ToF-MS Serum Analysis by HBI

Variable ID	Spearman Rank	p value	q value
985	-0.3114	0.0004	0.7632
1018	-0.2815	0.0013	0.776613486
1468	-0.246	0.0053	0.776613486
197	-0.2437	0.0058	0.776613486
1464	-0.2347	0.0079	0.776613486
1742	-0.2302	0.0092	0.776613486
1009	-0.2294	0.0095	0.776613486
1227	-0.228	0.0099	0.776613486
487	0.2261	0.0106	0.776613486
320	-0.2213	0.0124	0.776613486
1284	-0.2199	0.013	0.776613486
1641	-0.2183	0.0137	0.776613486
560	-0.2181	0.0138	0.776613486
622	-0.2114	0.017	0.776613486
394	-0.2098	0.0179	0.776613486
1368	0.2069	0.0196	0.776613486
1532	0.2054	0.0205	0.776613486
1059	-0.2041	0.0214	0.776613486
1206	-0.2033	0.0219	0.776613486
1764	0.2024	0.0225	0.776613486
1629	-0.2013	0.0232	0.776613486
983	-0.2002	0.0241	0.776613486
1421	-0.2001	0.0241	0.776613486
849	-0.1999	0.0242	0.776613486
1013	-0.1987	0.0252	0.776613486
1329	-0.1985	0.0252	0.776613486
1760	-0.1983	0.0255	0.776613486
1064	-0.198	0.0257	0.776613486
1292	0.1955	0.0276	0.776613486
493	-0.1935	0.0293	0.776613486
1460	0.1899	0.0325	0.776613486
619	-0.1896	0.0327	0.776613486
1182	-0.1894	0.033	0.776613486
1280	-0.1887	0.0336	0.776613486
1155	-0.1883	0.034	0.776613486
727	-0.1876	0.0347	0.776613486
1371	-0.1874	0.0349	0.776613486
755	-0.187	0.0353	0.776613486
1004	-0.1864	0.0359	0.776613486
534	-0.1859	0.0364	0.776613486
1750	0.1843	0.038	0.776613486
1504	0.1843	0.0381	0.776613486
338	-0.183	0.0394	0.776613486
688	-0.183	0.0394	0.776613486
769	-0.1828	0.0397	0.776613486
1225	-0.1827	0.0398	0.776613486
951	-0.1824	0.0401	0.776613486
1023	-0.1819	0.0406	0.776613486
1113	-0.182	0.0406	0.776613486
1179	0.1818	0.0408	0.776613486

1591	-0.1815	0.0411	0.776613486
1820	-0.181	0.0417	0.776613486
954	-0.1803	0.0425	0.776613486
1199	-0.1802	0.0427	0.776613486
1859	0.1801	0.0427	0.776613486
864	-0.1792	0.0438	0.776613486
1756	-0.1785	0.0446	0.776613486
1461	-0.1784	0.0448	0.776613486
1523	0.1781	0.0451	0.776613486
245	-0.178	0.0452	0.776613486
1050	-0.1769	0.0466	0.776613486
1223	0.1767	0.0469	0.776613486
352	-0.1762	0.0475	0.776613486
621	-0.1762	0.0475	0.776613486
1374	-0.1757	0.0481	0.776613486
1443	0.1753	0.0487	0.776613486
157	-0.1745	0.0497	0.776613486

All of the variables identified were very weakly or weakly associated with the HBI. None of the variables identified maintain significance in relation to the HBI following FDR correction (q value).

4.22.5 Experiment 20 Summary

HBI analysis did not reveal any significant associations between disease activity score and identified variables on either UHPLC-FTMS or GC-ToF-MS platforms for both urine and serum samples.

4.23 Ulcerative Colitis Extent of Disease and SCCAI

The following experiments, based upon the longitudinal group, aim to determine whether the extent of disease identified in the Paris Classification, and the disease activity score (SCCAI), have discernable metabolomic profiles.

4.23.1 Experiment 21: Ulcerative Colitis Extent of Disease Differentiation

In experiment 21 we consider the group of patients with ulcerative colitis, and aim to determine whether the extent of disease can be differentiated by the metabolic profile. The variables have been collected as part of the Paris Classification;

E1 ulcerative proctitis,

E2 Left-sided UC (distal to splenic flexure),

E2+ Left-sided UC (extending beyond the limitation of the endoscopic examination),

E3 Extensive (hepatic flexure distally),

E4 Pancolitis (proximal to hepatic flexure).

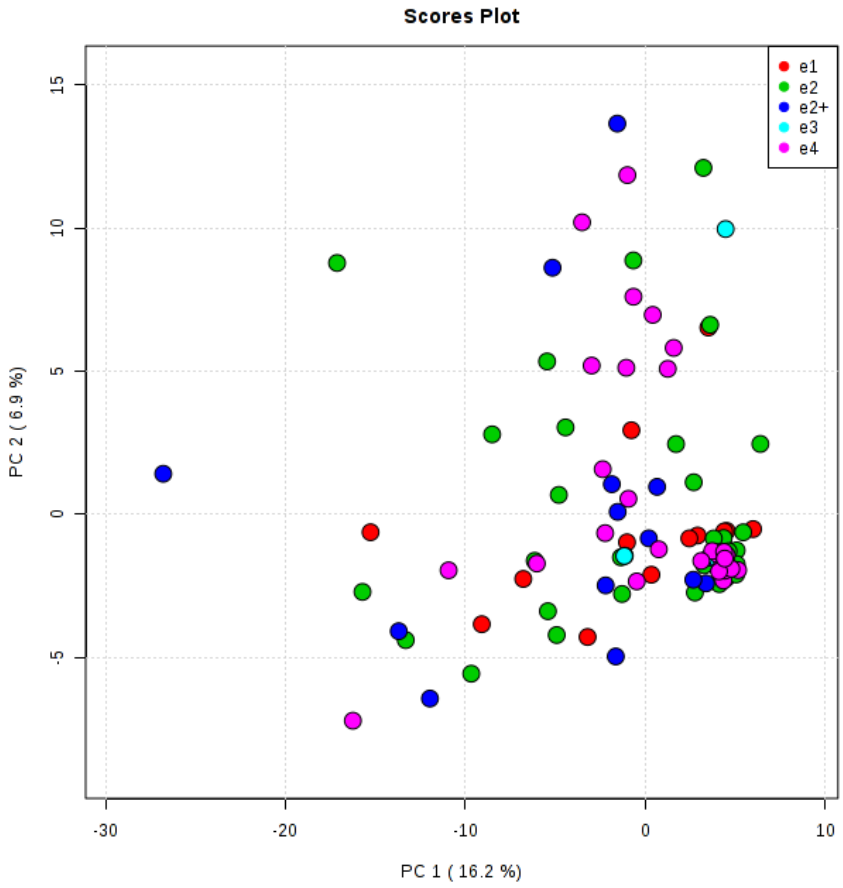
As in experiment 17 – 19, results are presented by means of principle component analysis plots.

Table 4.65 Experiment 21-22 number of samples analysed

	Ulcerative Colitis
Serum samples	127
Urine samples	125

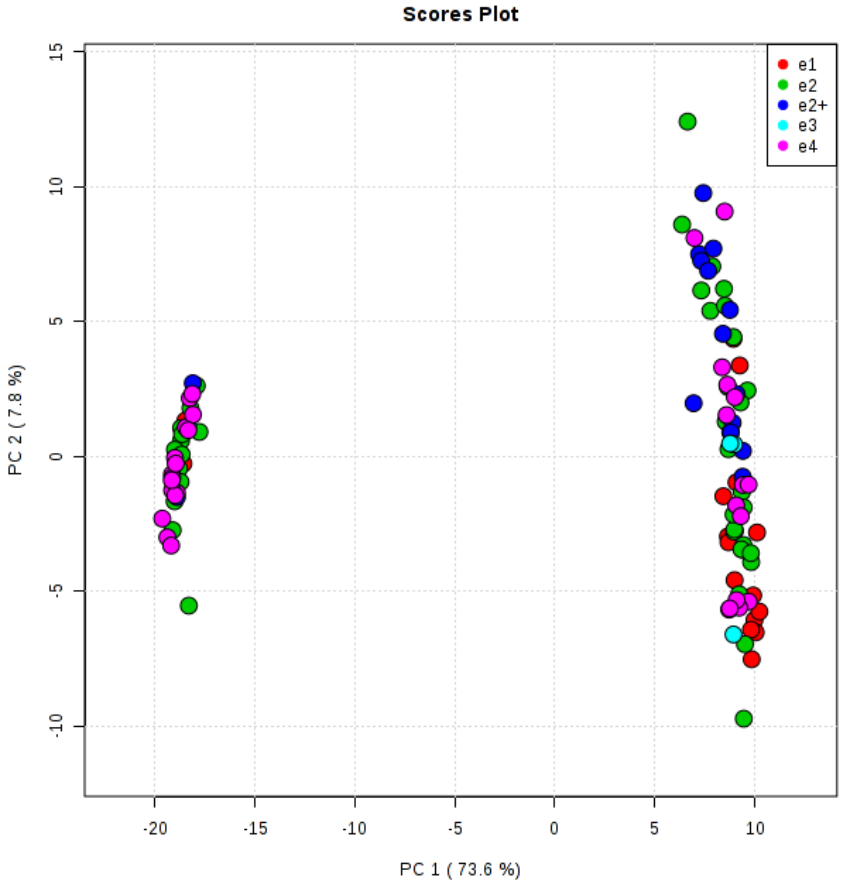
4.23.2 Experiment 21.1: Ulcerative Colitis Extent of Disease Differentiation Urine Analysis UHPLC-FTMS

Figure 4.129: PCA Plot UHPLC-FTMS Urine Analysis Grouped by Disease Extent



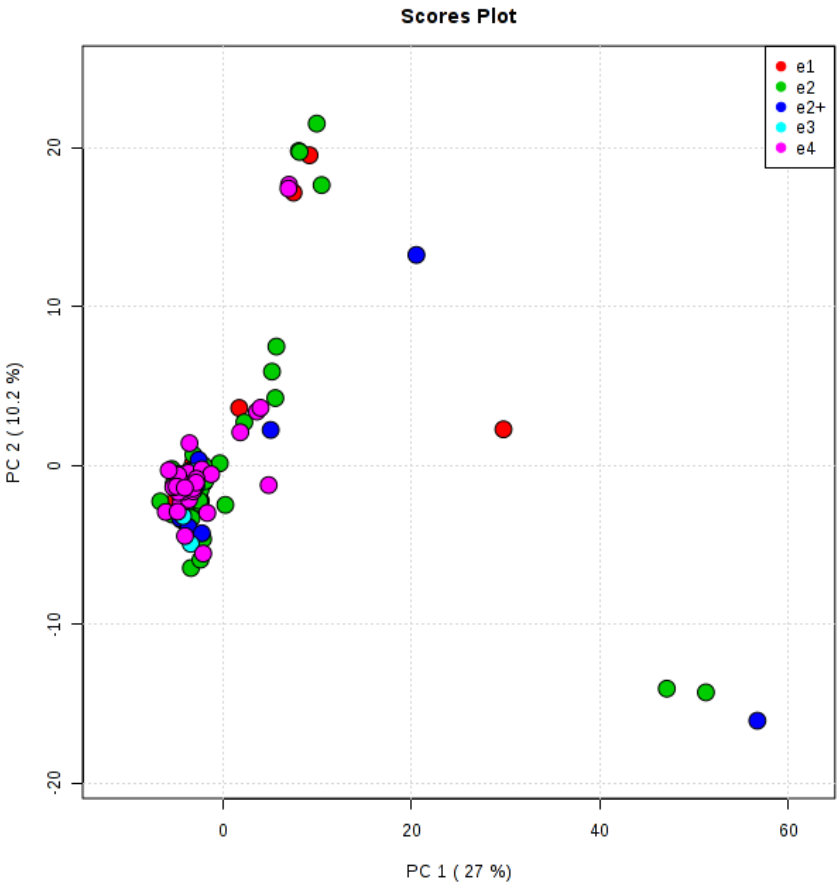
4.23.3 Experiment 21.2: Ulcerative Colitis Extent of Disease Differentiation Urine Analysis GC-ToF-MS

Figure 4.130: PCA Plot GC-ToF-MS Urine Analysis Grouped by Disease Extent



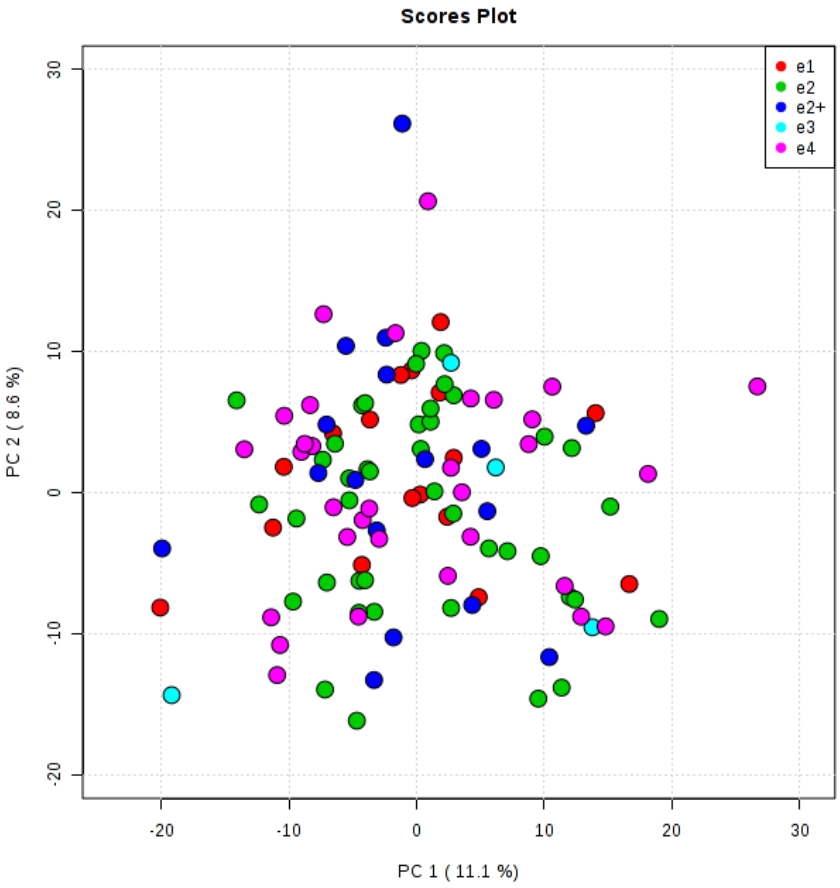
4.23.4 Experiment 21.3: Ulcerative Colitis Extent of Disease Differentiation Serum Analysis UHPLC-FTMS

Figure 4.131: PCA Plot UHPLC-FTMS Serum Analysis Grouped by Disease Extent



4.23.5 Experiment 21.4: Ulcerative Colitis Extent of Disease Differentiation Serum Analysis GC-ToF-MS

Figure 4.132: PCA Plot GC-ToF-MS Serum Analysis Grouped by Disease Extent



4.23.6 Experiment 21 Summary

In our study we have not been able to show differentiation between the metabolomic profiles of ulcerative colitis patients in relation to the disease extent, using either serum or urine samples, on UHPLC-FTMS or GC-ToF-MS platforms.

4.24 Experiment 22: Ulcerative Colitis Differentiation by SCCAI

In Experiment 22 we consider the group of patients with Ulcerative Colitis, and aim to determine whether the disease activity score (SCCAI) can be differentiated by the metabolic profile. As in experiment 19, Spearman's rank correlation coefficient is used to determine association, and FDR correction rather than Bonferroni is used.

In this experiment, variables were deemed significant if both the p value and the q value (FDR corrected) were <0.05.

4.24.1 Experiment 22.1: Ulcerative Colitis Differentiation by SCCAI Urine Analysis UHPLC-FTMS

Table 4.66: Important Variables Identified UHPLC-FTMS Urine Analysis by SCCAI

Variable ID	Spearman Rank	p value	q value
188	-0.29629	0.001592	0.464688
334	-0.29046	0.001984	0.28969
2	-0.28521	0.002412	0.234749
232	0.274366	0.003568	0.260406
277	-0.27252	0.003808	0.222351
15	-0.27116	0.003994	0.194335
187	0.266453	0.004702	0.196107
176	0.265454	0.004866	0.177579
304	-0.25685	0.006503	0.210944
90	0.255876	0.006717	0.196091
72	-0.24401	0.009856	0.261587
261	0.242461	0.010349	0.251786
178	0.23933	0.011412	0.256281
81	-0.23748	0.012082	0.251965
191	0.233673	0.013576	0.26423
308	-0.22895	0.015647	0.285505
245	0.22869	0.015769	0.270815
314	-0.22846	0.01588	0.257567
54	0.221457	0.019498	0.299612
25	0.217558	0.021807	0.31833
28	0.216799	0.022283	0.309787
173	-0.20936	0.027438	0.364114
315	-0.20822	0.028308	0.359333
250	0.204725	0.031132	0.378718
155	-0.2039	0.03183	0.371714
107	0.199955	0.035369	0.397154
125	0.196776	0.038454	0.415809
168	0.195812	0.039433	0.411162
123	-0.1945	0.040801	0.41076
31	-0.19446	0.040843	0.397479
249	0.193916	0.041419	0.390081
104	-0.1914	0.044181	0.403084
180	0.189023	0.046935	0.41524
189	0.188983	0.046983	0.403434
278	0.188525	0.047529	0.39646

327	-0.18792	0.048263	0.3914

All of the variables identified were very weakly or weakly associated with the SCCAI. None of the variables identified maintain significance in relation to the SCCAI following FDR correction (q value).

4.24.2 Experiment 22.2: Ulcerative Colitis Differentiation by SCCAI Urine Analysis GC-ToF-MS

Table 4.67: Important Variables Identified GC-ToF-MS Urine Analysis by SCCAI

Variable ID	Spearman Rank	p value	q value
133	-0.43547	1.78E-06	6.79E-04
30	-0.3442	0.000216	0.041225
326	-0.31263	0.000836	0.060962
281	-0.3068	0.001056	0.060962
204	-0.3046	0.001152	0.060962
36	-0.30093	0.00133	0.060962
113	-0.29793	0.001494	0.060962
223	-0.29766	0.00151	0.060962
82	-0.29636	0.001587	0.060962
14	-0.29615	0.0016	0.060962
70	-0.28874	0.002117	0.073309
184	-0.281	0.002814	0.089332
283	-0.2747	0.003526	0.091106
39	-0.27291	0.003757	0.091106
174	-0.27205	0.003871	0.091106
3	-0.27099	0.004017	0.091106
249	-0.27065	0.004065	0.091106
220	-0.26629	0.004728	0.100073
236	-0.26278	0.00533	0.100678
260	-0.26264	0.005356	0.100678
310	-0.26159	0.005549	0.100678
91	-0.25078	0.007936	0.131286
101	-0.24796	0.008691	0.131286
275	-0.24711	0.00893	0.131286
149	-0.24658	0.009083	0.131286
394	-0.24605	0.009239	0.131286
205	-0.24514	0.00951	0.131286
393	-0.24332	0.010074	0.131286
397	-0.24306	0.010157	0.131286
37	-0.2425	0.010337	0.131286
391	-0.23829	0.011785	0.136868
103	-0.23729	0.012155	0.136868
50	-0.23649	0.012456	0.136868
130	-0.23647	0.012465	0.136868
44	-0.23594	0.01267	0.136868
7	-0.23439	0.013282	0.136868
74	-0.23429	0.013322	0.136868
239	-0.23349	0.013651	0.136868
305	-0.2308	0.014808	0.144659
388	-0.22799	0.016102	0.14879
131	-0.22614	0.017008	0.14879
173	-0.22562	0.017268	0.14879
27	-0.22506	0.017554	0.14879
330	-0.22439	0.017902	0.14879
235	-0.22399	0.018113	0.14879
344	-0.223	0.018646	0.14879
68	-0.2229	0.018699	0.14879
222	-0.22216	0.019106	0.14879

278	-0.22211	0.019136	0.14879
264	-0.22087	0.019831	0.149815
98	-0.21961	0.020565	0.149815
215	-0.21887	0.021006	0.149815
323	-0.21833	0.021334	0.149815
398	-0.2174	0.021904	0.149815
43	-0.21689	0.022227	0.149815
52	-0.21613	0.02271	0.149815
200	-0.21599	0.022796	0.149815
261	-0.21575	0.022957	0.149815
160	-0.21486	0.023537	0.149815
309	-0.21337	0.024544	0.149815
42	-0.21236	0.025248	0.149815
234	-0.21216	0.025386	0.149815
338	-0.21195	0.02554	0.149815
401	-0.21147	0.02588	0.149815
116	-0.21141	0.025921	0.149815
38	-0.2112	0.026072	0.149815
300	-0.21065	0.026473	0.149815
245	-0.2099	0.027028	0.149815
288	-0.20932	0.027468	0.149815
77	-0.20919	0.027565	0.149815
53	-0.2085	0.028088	0.149815
399	-0.2081	0.028401	0.149815
356	-0.20732	0.029015	0.149815
21	-0.20707	0.029209	0.149815
241	-0.20667	0.029536	0.149815
221	-0.20623	0.029884	0.149815
5	-0.20541	0.030562	0.150146
364	-0.20506	0.03085	0.150146
315	-0.20443	0.031384	0.150146
78	-0.20426	0.031527	0.150146
20	-0.20296	0.032649	0.153573
2	-0.20081	0.034578	0.158321
343	-0.20067	0.034704	0.158321
23	-0.20045	0.034905	0.158321
216	-0.1994	0.035892	0.158577
154	-0.19876	0.036503	0.158577
318	-0.19869	0.036571	0.158577
118	-0.19863	0.036627	0.158577
167	-0.19617	0.039065	0.163111
231	-0.19554	0.039712	0.163111
363	-0.19521	0.040052	0.163111
402	-0.19428	0.041036	0.163111
279	-0.19426	0.041055	0.163111
54	-0.19404	0.041291	0.163111
327	-0.19397	0.041358	0.163111
161	-0.1932	0.04219	0.163111
151	-0.19308	0.042325	0.163111
121	-0.19272	0.042713	0.163111
304	-0.19247	0.042992	0.163111
11	-0.19209	0.04341	0.163111
317	-0.19162	0.043931	0.163111
166	-0.19152	0.044046	0.163111
372	-0.19148	0.044096	0.163111

292	-0.19071	0.044968	0.164739
163	-0.1899	0.045902	0.165824
90	-0.1897	0.046135	0.165824
203	-0.1889	0.047078	0.166565
126	-0.18879	0.047215	0.166565
66	-0.18825	0.04786	0.16708
320	-0.18784	0.048354	0.16708
319	-0.18745	0.048837	0.16708
259	-0.18722	0.049115	0.16708
117	-0.18674	0.049709	0.167604

Only variable ID 133 (moderately associated) and variable ID 30 (weakly associated) are significantly related to the SCCAI following FDR correction. They are both unknown metabolites.

4.24.3 Experiment 22.3: Ulcerative Colitis Differentiation by SCCAI Serum Analysis UHPLC-FTMS

Table 4.68: Important Variables Identified UHPLC-FTMS Serum Analysis by SCCAI

Variable ID	Spearman Rank	p value	q value
115	-0.2539	0.006414	1
123	0.236886	0.011161	1
81	-0.23646	0.011314	0.868529
142	-0.23407	0.01219	0.70187
336	-0.22619	0.015526	0.715166
35	0.216186	0.020878	0.801372
138	-0.21071	0.024427	0.803659
90	0.210203	0.024784	0.713479
79	-0.20179	0.031317	0.801374
247	-0.19449	0.03812	0.877929
169	-0.19369	0.038934	0.815163
170	-0.18929	0.043689	0.83848
243	-0.18587	0.047702	0.845082
93	-0.18429	0.049661	0.816937

All of the variables identified were very weakly or weakly associated with the SCCAI. None of the variables identified maintain significance in relation to the SCCAI following FDR correction (q value).

4.24.4 Experiment 22.4: Ulcerative Colitis Differentiation by SCCAI Serum Analysis GC-ToF-MS

Table 4.69: Important Variables Identified GC-ToF-MS Serum Analysis by SCCAI

Variable ID	Spearman Rank	p value	q value
904	-0.32232	0.00047	0.636686
886	0.295282	0.001426	0.965727
1788	-0.29281	0.001569	0.70884
1189	0.280751	0.002481	0.840365
1648	-0.27798	0.002749	0.74484
1731	-0.27369	0.003214	0.725826
907	-0.26672	0.004124	0.798165
606	-0.26238	0.0048	0.813013
1814	-0.25861	0.005466	0.822821
759	0.250557	0.007172	0.971701
723	0.249251	0.007489	0.922439
501	0.24355	0.009023	1
567	0.241833	0.009536	0.993891
23	-0.24057	0.009931	0.96109
387	0.238057	0.010756	0.971585
969	0.237242	0.011037	0.934626
1570	0.236747	0.011211	0.893497
892	0.231676	0.013131	0.988392
1589	-0.22956	0.014014	0.999351
264	-0.22704	0.015131	1
199	0.226184	0.015528	1
903	0.225791	0.015714	0.967772
438	0.225279	0.015958	0.940084
1482	0.222854	0.01716	0.968773
1787	0.222072	0.017564	0.951927
1475	-0.21929	0.019068	0.993702
1371	-0.21788	0.019874	0.997344
571	0.217276	0.020227	0.978769
1479	-0.21291	0.022948	1
802	-0.21239	0.023289	1
1004	0.207581	0.026682	1
664	0.207544	0.02671	1
1291	-0.20614	0.027782	1
816	0.205263	0.028463	1
1079	-0.20476	0.02886	1
658	-0.20451	0.029064	1
902	-0.20449	0.029077	1
613	-0.20339	0.029974	1
975	0.203006	0.030293	1
186	0.20256	0.030667	1
859	0.202417	0.030787	1
624	0.202273	0.030909	0.997113
1880	0.202179	0.030988	0.97644
974	0.201704	0.031394	0.966731
1761	0.201507	0.031563	0.950338
1196	-0.20068	0.032279	0.950777
1869	0.199689	0.033163	0.956039
1644	-0.19966	0.033193	0.936953

998	0.199529	0.033307	0.920994
622	0.199492	0.03334	0.903475
429	0.199357	0.033463	0.889006
682	-0.19893	0.033854	0.882106
451	0.198821	0.033951	0.867955
534	-0.19702	0.035638	0.894189
900	0.196572	0.036066	0.888485
1387	0.196089	0.036535	0.883959
57	0.195913	0.036707	0.87254
737	-0.19564	0.036976	0.863791
611	0.194946	0.037663	0.864928
1241	-0.19467	0.037943	0.856823
586	-0.19459	0.038021	0.844518
835	0.193525	0.039107	0.854639
1713	-0.19331	0.039328	0.845813
945	0.191588	0.041151	0.871187
923	0.191432	0.041318	0.861284
1360	-0.19132	0.041438	0.850692
1610	0.191199	0.041571	0.840691
159	0.190506	0.042329	0.843425
1571	-0.18751	0.045741	0.898191
1667	0.187472	0.045789	0.886293
906	0.187349	0.045934	0.876575
1491	0.186812	0.046571	0.876389
30	0.186276	0.047215	0.876345
1642	0.18603	0.047513	0.869951
548	0.185825	0.047762	0.862854
714	0.185416	0.048264	0.860444
1893	0.185207	0.048521	0.853801
256	-0.18512	0.048633	0.84479
1228	0.184957	0.048831	0.837494
316	0.184895	0.048907	0.828318
358	0.184203	0.049774	0.832597

All of the variables identified were very weakly or weakly associated with the SCCAI. None of the variables identified maintain significance in relation to the SCCAI following FDR correction (q value).

4.24.5 Experiment 22 Summary

In this experiment only 2 statistically significant variables were identified, both during analysis of urine samples on the GC-ToF-MS platform. Unfortunately they are both unknown metabolites. Analysis of serum and urine on the UHPLC-FTMS platform and serum on the GC-ToF-MS platform did not identify any significant metabolites in relation to the SCCAI.

5

Discussion

5.1 Results Overview

This is the first study in human IBD in which paired and longitudinal sampling methodologies have been employed to investigate metabolomic profiles in various disease states and in relation to certain treatments. The utilisation of two biofluids, serum and urine, on UHPLC-FTMS and GC-ToF-MS analytical platforms allows us significant insight into the human metabolome in IBD, and is therefore a useful discovery study.

5.2 Comparison to Previous Work: UC v CD v HC

The initial experiments carried out in our study allow us to make a comparison with previous work carried out by the group working under Professor Goodacre in Manchester Institute of Biotechnology (Johnston 2014). By following the same rigourous sample collection and preparation procedures, it is possible to draw comparisons between their study and my own, although the previous study was carried out purely on a male population to avoid hormonal variation, and used single sample sets rather than paired or multiple sets of samples. Johnston et al identified the following classes to show most differentiation between healthy controls and IBD patients; Vitamin D and its metabolites, steroids and their derivatives, fatty acids, bile acids, phospholipids and phosphocholine. They specifically found that sphingolipids were exclusively decreased within the CD cohort compared to the control group and isoprenoids were decreased in the UC cohort compared to controls.

Interestingly, despite my cohort of patients being from a region significantly further North in the UK than the cohort studied by the Manchester group, vitamin D and its metabolites was not identified as a significant descriminant between IBD patients and HCs. A feasible explanation for this may be reduced levels throughout the entire study population including the HCs, thus no differentiation was identified. There is growing evidence that vitamin D may play a role in IBD pathogenesis, and potentially may have a therapeutic function (Hlavaty, Krajcovicova et al. 2015). An interesting follow up to both of these studies would be a direct comparison of the metabolomic profiles of IBD patients in the North of Scotland and IBD patients in the North of England, specifically searching for vitamin D and its metabolites.

In our study, we identified metabolites in the classes; fatty acyls, carboxylic acids and derivatives, carbohydrate and carbohydrate conjugates to be reduced in IBD compared to HCs.

The following specific metabolites have been identified as of biological relevance in our study: N-Acetylglutamine, Ethyl 1-(propylthio)propyl disulfide, Butyl 1-(methylthio)propyl disulfide, 4-Thiocyanatophenol, 5-aminoimidazole, imidazole-4-acetaldehyde, alternariol, hydroxyl-alpha-sanshool, propanetricarboxylic acid, threonine, dihydroxybutanoic acid, serine, fructose, aminomalonic acid, glucaronic acid, pentanoic acid, galactose, cellobiose and myo-insitol.

5.3 IBD Surgery Patients

This is the first study in human IBD metabolomics to consider patients undergoing surgical management of their disease, and the effects that may be translated into their profile. During the various experiments carried out on this patient group, we were able to utilise both grouped and paired

sample analyses.

Initially we compared pre and post surgical patients, and found that we were unable to differentiate between their profiles using either serum or urine samples, on either the GC-ToF-MS, or the UHPLC-FTMS platforms. We were, however, able to differentiate between surgical IBD patients and healthy controls. Urine samples showed more significant differentiation than serum samples, and urine samples analysed on the UHPLC-FTMS platform were the most significantly differential of all. Specific metabolite identification revealed cholesterol, 5-beta-cholestan-3-one, lathosterol, erythritol, propanetricarboxylic acid, 3'-hydroxy-3,4,5,4'-tetramethoxystilbene, biliverdin, N-Acetylglutamine, ethyl 1-(propylthio)propyl disulfide, butyl 1-(methylthio)propyl disulfide and 4-thiocyanatophenol, 5-aminoimidazole, 12,13-DHOME, 9,10-DHOME, methyl-cysteine, cysteinyl-methionine, 8-hydroxyguanosine, myricetine 3,3'-digalactoside, octanoic acid, citric acid, oleic acid, uric acid, retinyl ester, 2-arachidonylglycerol, monoacylglycerides and glycerol to be of relevance.

Metabolite identification experiments showed metabolites in the classes organic disulphides, benzene and substituted derivatives, carboxylic acids and derivatives, azoles, and fatty acyls to be decreased in both urine and serum samples of pre surgery IBD patients in comparison to healthy controls. Metabolites in the class carbohydrates and carbohydrate conjugates are increased in the urine of pre surgery IBD patients compared to healthy controls.

In post surgery IBD patients, urinary metabolites belonging to the super class alkaloids and derivatives, and the classes organic disulphides, benzene and substituted derivatives, carboxylic acids and derivatives, azoles, and 2-arylbenzofuran flavonoids are reduced when compared to healthy controls. Metabolites in the class prenol lips and glycerolipids are increased in the urine of post surgical IBD patients when compared to healthy controls.

5.4 Biological Therapy Patients

Again, this is a novel study in human IBD metabolomics, to consider patients undergoing biological therapy for their disease, and how their metabolomic profile may be affected by this treatment. During the various experiments carried out on this patient group, we were able to utilise both grouped and paired sample analyses. Initially we aimed to determine whether we could differentiate between pre and post biological therapy patients. We did not find any significant difference between the metabolomic profiles of pre- and post-biological therapy IBD patients when comparing either serum or urine samples on either UHPLC-FTMS or GC-ToF-MS platforms. We also found that we were not able to differentiate IBD patients undergoing biological therapy from healthy controls using either biofluid on either analytic platform.

Metabolite identification experiments showed higher levels of urinary metabolites in the class carboxylic acid and derivatives in pre biological therapy IBD patients compared to both post biological therapy IBD patients and healthy controls. In the post biological therapy IBD patients, higher levels of urinary fatty acyls and imidazole ribonucleosides and nucleotides were identified, as well as increased serum fatty alcohols, in comparison to healthy controls.

Specific metabolite identification revealed calystegine A3, 3-dehydrocarnitine, N-acetylvaline,

acetamide, lactose, 8-hydroxyguanosine, myricetin 3,3'-digalactoside, propanetricarboxylic acid, glycine, s-aminomethylduhydrolipoamide, phenylalanyl-arginine, arginyl-phenylalanine, pyrrolidonecarboylic acid, pyroglutamic acid, imidazoleacetic acid riboside, and *ortho*- and *para*-hydroxyatorvastatin to be of biological interest in IBD.

Interesting, Johnston at el (Johnston 2014) showed lower levels of carnitine in CD patients compared to healthy controls. In this study we showed increased levels of urinary 3-dehydrocarnitine, an intermediate in carnitine degradation, in pre biological therapy IBD patients compared to post biological therapy patients. Carnitine has anti-oxidant properties, reducing myeloperoxidase and malondialdehyde, and upregulating superoxide dismutase preventing the reduction of glutathione. It inhibits lipid peroxidation of phospholipid membranes therefore suppressing the formation and activation of reactive oxygen species, and inhibiting the NF-κB pathway. Carnitine is required for β-oxidation and therefore carnitine transporters, coded for by OCTN genes, have been implicated in IBD susceptibility, with reduced carnitine absorption leading to impaired fatty acid oxidation in intestinal epithelial cells, and cell injury (Moeinian, Farnaz Ghasemi-Niri et al. 2013). The increased urinary secrection in pre biological therapy IBD patients we have identified may be an indication that carnitine is not being utilised well in patients with active IBD.

Recently, in a rat IBD model, the combined effects of treatment with butyrate, *Lactobacillus casei* and L-carnitine showed a significantly beneficial effect in the alleviation of colitis (Moeinian, Ghasemi-Niri et al. 2014). Further investigation of this substance in relation to IBD is warrented in view of its significance in two separate metabolomic IBD studies, and its potential to translate into therapeutic clinical practice.

5.5 Treatment Naïve Patients

In this study, the group of treatment naïve patients was very small and thus any conclusions drawn must be interpreted with care. Specific metabolite identification deemed phosphate, peroxynitrite, nucleoside triphosphate, indane, di-hydroxymelatonin, acetyl-N-formyl-5-methoxykynurenamine, tracheloside, hippuric acid, adrenochrome, hydroxylamine and cellobiose of biological importance. Metabolites in the class non-metal oxoanionic compounds were found to be increased in the serum of drug naïve IBD patients pre treatment compared to post treatment. Serum metabolites in the class benzenes and substituted derivatives, and urinary metabolites in the class carbohydrates and carbohydrate conjugates, indoles and derivatives, and benzene and derivatives are reduced in drug naïve IBD patients compared to healthy controls. Metabolites in the class homogenous other non-metal compounds are increased in the urine of treatment naïve IBD patients compared to healthy controls.

5.6 Longitudinal Sampling Patients

This is the first study in IBD and metabolomic profiling to collect longitudinal biofluid samples and use participants as their own internal controls. Meticulous collection of patient phenotype, and disease activity score at each sampling episode was imperative to allow appropriate comparisons to be drawn.

This research methodology, which was carefully constructed in collaboration with Professor Roy Goodacre and Dr Warwick Dunn, has allowed the opportunity to study the effects on the metabolomic profile of disease behaviour, location and age at diagnosis in Crohn's disease patients (Montreal Classification), and of disease extent in ulcerative colitis patients (Paris Classification). In both CD and UC patients, disease activity scores, HBI and SCCAI respectively, were calculated at each sampling episode, and the effects on the metabolome established.

In Crohn's disease patients, it was not possible to show differentiation between the metabolomic profiles in relation to the disease behaviour displayed, the location of disease, or the age at diagnosis, using either serum or urine samples, on UHPLC-FTMS or GC-ToF-MS platforms. In relation to the disease activity score, it was not possible to identifiy any significant associations between metabolite variables and HBI.

In ulcerative colitis patients, we did not show any differentiation between the metabolomic profiles in relation to the disease extent, using either serum or urine samples, on UHPLC-FTMS or GC-ToF-MS platforms. In relation to the disease activity score, only two metabolite variables identified were significantly associated with the SCCAI. These were both identified in urine analysis on the GC-ToF-MS platform. Unfortunately, both of these metabolites are unknown variables.

It is not surprising that non-localised biofluids do not show alterations in the metabolome when considering potentially systematic factors such as disease behaviour and location. Further work on this subject could involve metabolomic analysis of location specific and behaviour specific tissue in order to determine potential alterations in the metabolome in relation to these factors.

5.7 Study Strengths

This study was designed in collaboration with the team from MIB, who have a plethora of experience and knowledge in the design and completion of metabolomics experiments. The study was designed to minimise bias and confounding factors, within the limitations of a clinical setting, whilst upholding the ethical requirements set. The strengths of this study are that it is case-controlled, and also uses participants as their own internal controls in certain experiments. A strict participant recruitment and sampling process was adhered to throughout the study, and all samples were taken in a single centre. The samples were prepared and analysed using validated protocols from MIB. Sample preparation was carried out by me under the supervision of Dr Drupad Trivedi and Dr Nicholas Rattray. Sample analysis on the GC-ToF-MS and UHPLC-FTMS platforms was carried out by the above experienced members of the MIB team to minimise experimental error.

All meta-data was collected prospectively by me to minimise reporting bias. The bioinformatics analysis was carried out by Dr Drupad Trivedi and Dr Yun Xu at MIB. I met with them on a regular basis to direct the clinical relevance of the analysis.

5.8 Study Limitations

As previously discussed, metabolomics involves the study of numerous analytes that have very diverse physical and chemical properties, and occur in a wide concentration range. Therefore,

currently no single analytical platform exists that allows for analysis of a complete profile. The metabolomic profile is also potentially very susceptible to alterations in environment and diet, and whilst patient and disease phenotype can be recorded, these variables are challenging to identify and correct for during studies.

Throughout this study, dietary and drug metabolites were identified. Whilst in the setting of IBD dietary variability may have some relevance, it is potentially a confounding factor. We attempt to minimise these effects by taking samples in the fasting state (>6 hours), and by recording dietary variations.

In this study most of the patients were receiving out-patient treatment. Those who were inpatients in the hospital will have had different dietary intakes from those eating in their own home. Similarly, patients requiring emergency surgery for conditions such as obstructive strictures will not have had the same pre-operative diet to those having an elective surgical procedure such as a completion proctectomy.

The medications taken throughout the study are identified in metabolomic profiles. Whilst a detailed drug history was taken from each of the participants, these findings are potentially confounding factors. It is challenging to control for medications as patients will be prescribed them for good reason and it is not ethical to stop them. In order to try and assess the profiles of treatment naïve IBD patients without drug contamination of their metabolomic profile, we attempted to recruit treatment naïve patients. The practicality of this was very challenging, as most patients identified with a possible new diagnosis of IBD were commenced on treatment in the primary care setting prior to contact with hospital services. Those who were identified as “treatment naïve” may have been on other non-IBD medications such as analgesics, anti-spasmodics, or may have received bowel preparation prior to their diagnostic endoscopic procedure.

The other recruitment challenge with this group of patients was that following a diagnosis of IBD being made, medical treatment should be commenced immediately. It was not ethical to delay treatment to allow for the 6 hour fasting window required to take samples for metabolomic analysis. This lead to a very small number of treatment naïve patients being recruited. A larger study population would be required to confidently assess the metabolomic profiles of treatment naïve IBD patients. The paediatric IBD population potentially offers a “cleaner” profile due to less confounding factors such as coexisting diseases and medication use, and the ability to evaluate the environment in which the child has spent most of their life.

In the surgical group, patients underwent different procedures for different conditions, ranging from perianal procedures, to colectomies. It is unknown how the physiological effects of major surgical intervention affect the metabolomic profile. We chose to sample patients 8 weeks post procedure as we would expect them to be back to near normal by then, and also to fit in with clinical appointments; however this time frame has not been evaluated in the metabolomics setting.

Ideally, standardisation of the surgical group would have involved chosing either CD or UC patients, and selecting one procedure to study. Investigation of patients with severe UC requiring colectomy would be a good group to study, as the operative procedure should rid the patient of the majority of

their disease burden, and thus allow useful comparison of a profile in the presence of severe disease compared to a profile that theoretically should be normalising.

Similarly, patients undergoing biological therapy combined both CD and UC patients, and treatment with both Infliximab and Adalimumab.

Standardisation of both the surgical and biological groups in future studies would allow a more homogenous group of profiles to be studied.

Both disease activity scoring systems (HBI and SCCAI) have limitations. They both rely on a score being created from the stool frequency reported by the patient. Should a patient have a stoma, the score is difficult to interpret. In these cases we simply used the patients' normal bowel frequency to correlate with a minimum score, and extrapolated the score from the extra frequency of stoma movement they reported.

Both scoring indices are reasonably subjective, with a score for "general well being" contributing significantly to the final result. It is well documented that levels of anxiety and depression are higher in IBD patients than in HCs (Mikocka-Walus, Knowles et al. 2016). Subjective scoring systems are at risk of reporting bias, and may not correlate well with physiological disease activity.

5.9 Advantages of UHPLC-FTMS and GC-ToF-MS

As previously discussed, the human metabolome is vast. The diversity encountered does not lend itslf to a single platform method of analysis. The use of two platforms, GS-ToF-MS, and UHPLC-FTMS allows a more complete spectrum of metabolites to be analysed, as does the use of more than one biofluid. This is especially relevant in this discovery type study, where we aimed to identify a quantitive compliment of metabolites in relation to each disease, and disease course.

One of the limitations of GC-MS is that only volatile compounds, or those that can be made volatile by chemical derivatisation, can be analysed. The derivatisation process has the potential to be a confounding factor in the present study. GC, however, can identify up to 200 metabolic features in a serum or plasma sample, and provides separation of molecules at low molecular weight (18 – 350Da), allowing identification of metabolite classes such as amino acids, amines, amides, and sugars.

The UHPLC-FTMS platform used within this study allows for high mass resolution and high mass accuracy (typically 5 p.p.m), and provides high chromatographic resolution with high mass accuracy, that is used for putative metabolite identification.

Unlike GC-MS, which uses capillary columns that provides appropriate chromatographic resolution with peak widths of 2 – 5 seconds and reproducible retention times, in LC-MS retention time and collision-induced dissociation mass spectra are not reproducible between systems due to differences in LC column chemistries, and therefore mass transferable mass spectral libraries are not available.

In UHPLC-FTMS, samples are analysed in positive and negative ion mode as they provide completentary data, and some metabolites will only be detected in a single-ion mode, dependent on their charge.

The combination of GC-ToF-MS and reversed phase UHPLC-FTMS is well accepted, and provides the opportunity to detect low-molecular weight molecules with a boiling point low enough to allow

elution through a GC column, and higher molecular weight compounds including many lipid classes, at very low concentrations respectively. Unfortunately, as many of the variables identified using chromatography and mass spectroscopy are as yet “unknown”, putative metabolite identification is challenging, and will remain this way until the human metabolome is more fully, if not completely, characterised.

The use of NMR may be able to provide better quantitative accuracy of targeted metabolites, and is worth considering in future studies.

5.10 Optimisation and Planning of Future Studies

This is a small study, and the results are potentially limited by low sample numbers and patient heterogeneity within each of the groups analysed.

5.10.1 Patient Selection

In order to optimise results patient cohorts should be as closely grouped as possible, including sex (ideally male to avoid changes in hormone levels), age, social and dietary habits, area of habitation, past medical history, and medication usage. In clinical studies these variables are difficult to control for and therefore potentially need to be taken into account.

5.10.2 Patients Undergoing Biological Therapy

A longitudinal study with biofluid samples taken at each drug administration out to 1 year, rather than just the initial loading of the medication, may show alterations in profile. Comparing patients with one biological therapy and either UC or CD, rather than a combination of both, would provide a more homogenous study group.

5.10.3 Patients Undergoing Surgery

Ideally as many confounding factors as possible should be alleviated in order to achieve as pure a profile as possible. In order to do this, future studies could focus on one disease process and one operative treatment. UC is less heterogeneous than CD as it only affects the large bowel. The surgical procedure of choice is also more standardised than in CD and therefore UC patients undergoing subtotal colectomy would be a sensible study medium. In order to reduce “background noise” the group selected should ideally have had the same treatment for their disease and over a similar timeframe. It may be that selecting patients with an acute flare of severe colitis that come to require surgery is optimal. If pre-operative medical management is standardised and these patients are identified at first presentation, metabolomic profiles should be the most homogeneous. Of course in clinical practice this is unlikely to be achievable and therefore meticulous recording of disease activity, medications, and if possible diet, should be mandatory when carrying out ‘omics studies.

5.10.4 Biofluid Choice

Blood and urine are readily available biofluids ameanable to metablomic analysis. Sample collection

is minimally invasive for blood and non-invasive for urine, and therefore these integrative biofluids that incorporate the functions and phenotypes of many different parts of the body in a single sample, are attractive biofluids to study. However, the "metabolomic footprint" identified is a complex combination of many bodily functions, and hence teasing out relevant information from potentially thousands of metabolites can be challenging (Dunn, Broadhurst et al. 2011).

Faecal samples provide another non-invasive sampling methodology, with the advantage over urine and serum that the sample has been in the gut lumen and in contact with the mucin layer on the gut mucosa. Therefore faecal samples may provide insights into the gut microbiome and disease pathogenesis not offered by other biofluids. However, the collection of faecal samples, and the transportation and storage, as well as processing provides more challenges than serum and urine.

Tissue samples are considered the gold standard in clinical practice in the diagnosis of IBD, however the collection of tissue samples is an invasive procedure and few metabolomic studies using human IBD gut tissue have been carried out. Groups have however shown differentiation between IBD and HCs (Balasubramanian, Kumar et al. 2009), active and quiescent UC (Bjerrum, Nielsen et al. 2010), although other authors have reported similarities between these groups (Sharma, Singh et al. 2010).

When considering future studies the utilisation of faecal samples and tissue samples must be considered. However, it is likely that a combination of biofluids and tissue samples may in fact provide the most robust and accurate profile, although the analysis of such a large group of variables would be extremely challenging.

5.10.5 Focus on Specific Metabolite Classes

Throughout our experiments, biologically relevant metabolites from the classes carboxylic acids and derivatives, benzene and substituted derivatives, fatty acyls, and azoles appear to be the most prevalent. A focused study of these particular metabolite groups may yeald interesting results with regards to disease pathogenesis and biomarker discovery.

5.11 Future IBD Studies

To allow a complete analysis of the human metabolome in IBD, a systems-wide approach utilising multiple biological samples, ideally taken at a single sitting, could be undertaken. Sampling of blood, urine, tissue, faeces and colonic mucus may lead to futher understanding of disease pathogenesis. Ideally multiple platforms (GC-MS / LC-MS +/- NMR) would be utilised to gain knowledge of as wide a metabolome as possible.

In the search for biomarkers to assess and monitor disease activity, using patients as their own controls seems important. It may be that a more objective measure of disease severity is required, than the subjective disease activity scores that we use at present, to identify metabolites that differentiate disease severity. Objective findings such as histological results in resectional specimens or tissue biopsies may prove useful in identifiying corresponding differentiating metabolites in biofluid samples.

In all metabolomics studies, environmental factors can prove be very challenging to both control for

and consider during analysis. The paediatric population may offer an opportunity to study patients with less confounding factors than adults. Many paediatric IBD patients will be treatment naïve prior to diagnosis, and will not have the underlying disease processes present in large numbers of the adult population, such as atherolscleortic disease or diabetes. They are also less likely than an adult population to have been exposed to social factors such as tobacco and alcohol, and are more likely than adults to have lived in one place for their whole life. Studying this population may give a "cleaner" metabolomic profile than in an adult, although other factors such as growth hormones and their effect on the metabolome would have to be considered.

Finally, there is great potential gain knowledge of disease pathogenesis from treatment naïve patients. In this study it proved incredibly challenging to recruit and sample these patients prior to a treatment regimen commencing. Larger numbers than were studied here are required to draw any conclusions regarding metabolomic profile. It may be that recruitment in the primary care setting would be necessary; however, logistically this would be a very difficult study.

5.12 Challenges Faced

This study was the first of its kind carried out from Raigmore Hospital, which lead to a number of challenges. I was heavily involved in the initial set-up of my work area within the Centre for Health Science. This involved the networking of computers onto the NHS system, and the procurement of a -80ºC freezer and all of the equipment required for the preparation and storage of biofluid samples.

As there was not a longstanding research network in this area I spent time discussing the project with both the general surgical and gastroenterology teams in Raigmore Hospital. I also sent information to all of the general practitioners in the region, and visited those who requested a meeting or further information about the project.

In a setting new to the recruitment of patients into clinical studies, I found that altering the mindset of clinicians to consider everyone as a potential participant into the study was challenging. This required a near constant presence from me at clinics and within the department of gastroenterology to ensure the potential for recruitment was optimised. There were similar difficulties with healthy controls, where clinical staff were sometimes unwilling for a patient to have the opportunity to spend time with me to take samples in case it delayed their journey to theatre.

Working on a project that required complete recruitment and sample collection before it was possibleto start the experiments was frustrating at times. Whilst the expertise from the team at MIB is invaluable and this project could not have succeeded without collaboration, I found it difficult to wait for my time on the analysers, and the delays seen with machine failures and then complex data analysis were reasonably lengthy. This lead to a prolonged project duration, with results only becoming available for analysis at the very end of the allocated timeframe.

6

Conclusion

6 Conclusion

Inflammatory bowel disease comprises two main disease subtypes; Crohn's Disease, and ulcerative colitis. Both are relapsing, remitting conditions, with significant socioeconomic implications.

The diagnosis and monitoring of these disease processes can be challenging, and currently there are no biomarkers in routine clinical practice.

The pathogenesis of IBD is complex and is not fully understood. Genetic, immunological and microbiological studies have provided great insights, and the use of the "omics" technologies have the potential to further advance our understanding.

This thesis describes a novel metabolomics profiling study. I aimed to identify urinary and serum metabolites that differentiate between IBD and healthy controls, and also to interrogate the differences in metabolomic profiles during different phases of disease, with special consideration given to the effects of medical and surgical treatments. In order to do this, patients were recruited into a longitudinal sampling group, a surgical group and a biological therapy group.

To optimise of the metabolomic profile identified, serum and urine samples were studied on both GC-ToF-MS and UHPLC-FTMS platforms. The biofluid selected were chosen as samples can be obtained using minimally invasive methods, and as such they have the potential to be utilised in future clinical practice.

The study carried out identified metabolites in the classes; benzene and substituted derivatives, carboxylic acids and derivatives, organic disuplphides, azoles, and fatty acyls to be of most relevance in delineating between the disease phenotypes. Analysis of the specific metabolites identified has given insights into the metabolic disturbances that occur in these diseases, and the effects of medical and surgical treatments.

It was not possible to identify specific metabolites that would differentiate disease activity score or disease behaviour or location in either CD or UC in this relatively small discovery study.

Future research should include larger population studies, ideally of the most hetrogenous groups possible. Utilising paired or longitudinal sampling techniques allows patients to act as their own internal controls, and is important when considering technologies such as metabolomics, which are strongly affected by environmental factors. Drug naïve IBD patients are a specific group that should be concentrated on as they may give the truest representation of the IBD metabolome, without the contamination of treatment regimens. Focus on specific metabolite classes that seem to differentiate between disease phenotypes should be considered in the search for biomarkers.

7

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8

Appendices

8.1 North of Scotland Ethic Committee Approved Study Documentation

NRES Committees - North of Scotland
Summerfield House
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19 May 2011

Mr Angus J M Watson
Consultant General and Colorectal Surgeon
NHS Highland
Department of Surgery
Raigmore Hospital
Old Perth Road
INVERNESS
IV2 3UJ

Dear Mr Watson

Study title: **Metabolomics and inflammatory bowel disease**
REC reference: **11/AL/0238**

The Research Ethics Committee reviewed the above application at the meeting held on 12 May 2011.

Ethical opinion

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Ethical review of research sites

NHS Sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Other conditions specified by the REC

- A6-2 – the Committee noted that the first approach to participants will be by the clinician responsible for the participant's care or by the IBD specialist nurse. Please confirm that the IBD specialist nurse will approach participants as part of the clinical team and not as part of the research team.

- A21 – the Committee noted that participants would be in the study for 3 years, however elsewhere in the paperwork it states 12 months. Please clarify.

- Participants Information Sheets – please remove 'Please hand this to the reception staff at the clinic when you arrive for your appointment' from all of the Information Sheets. Please forward revised copies of the Information Sheets.

- Participant Information Sheet – Controls – please remove the heading 'Will my GP be informed?' and the paragraph below as this is not required. Please forward a copy of the amended Information Sheet to the Committee.

- The Committee noted that in your paperwork, you are listed as Mr Angus Watson and Professor Angus Watson. The Committee ask that you are consistent in your title. Please forward copies of the amended documents.

It is responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

You should notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. Confirmation should also be provided to host organisations together with relevant documentation

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*	

Covering Letter		18 April 2011	
GP/Consultant Information Sheets: Sheet 1	2	7 April 2011	
Investigator CV: Angus Watson		20 April 2011*	
Letter of invitation to participant: Metabolomics and IBD Patient	2	7 April 2011	
Other: Unfavourable Opinion Letter		16 March 2011	
Other: Investigator CV: Diane Hildebrand		18 April 2011	

Document	*Version*	*Date*	
Other: Metabolomics and IBD Consultant Letter	2	7 April 2011	
Other: Consultant Information Sheet	2	7 April 2011	
Other: Metabolomics and IBD GP Letter	2	7 April 2011	
Other: Metabolomics and IBD GP Letter 2	2	7 April 2011	
Other: GP Information Sheet - Sheet 2	2	7 April 2011	
Other: Metabolomics and IBD GP Letter 3	1	7 April 2011	
Other: GP Information Sheet - Sheet 3	1	7 April 2011	
Participant Consent Form: Control	2	7 April 2011	
Participant Consent Form: IBD	2	7 April 2011	
Participant Information Sheet: IBD Quarterly	1	7 April 2011	
Participant Information Sheet: IBD Surgery	1	7 April 2011	
Participant Information Sheet: IBD Biological	1	7 April 2011	
Participant Information Sheet: Control	2	7 April 2011	
Protocol	2	7 April 2011	
REC application	79685/208112/1/982	21 April 2011	

* date received

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Service website > After Review.

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nres.npsa.nhs.uk.

11/AL/0238 Please quote this number on all correspondence

With the Committee's best wishes for the success of this project

Yours sincerely

Dr John Callender
Acting Vice-Chair

Enclosures: List of names and professions of members who were present at the meeting and those who submitted written comments
"After ethical review – guidance for researchers"

Copy to: Miss Frances Hines, NHS Highland

North of Scotland Research Ethics Committee (2)

Attendance at Committee meeting on 12 May 2011

Committee Members:

Name	*Profession*	*Present*	*Notes*	
Mr Stuart Bale	Lay Member - Retired HSE Manager - Shell	No		
Dr Jennifer Caldwell	Senior Lecturer in Occupational Therapy	No		

Dr John Callender	Associate Medical Director	Yes		
Dr Sarah Christie	Lay Member - Reader in Law	Yes		
Dr Medhat Ezzat	Consultant Neonatologist	Yes		
Dr Georgina Hold	Senior Lecturer - Gastroenterology	No		
Miss Rhoda MacKenzie	Clinical Teaching Fellow - Vascular Surgery	Yes		
Dr Mandy Moffat	Research Fellow - Psychology	Yes		
Dr Jeremy Morse	Manager of Clinical Skills	Yes		
Mr Alistair Ritchie	Lay Member - Retired Police Officer - Grampian Police	Yes		
Dr Andy Schofield	Senior Lecturer in Surgery & Molecular & Cell Biology	No		
Dr Ruth Stephenson	Chair and Consultant in Anaesthesia	No		
Mrs Juliette Watson	Quality & Training Manager	Yes		
Mrs Fiona Watson	Lay Member - Ex Company Director	No		

Also in attendance:

Name	*Position (or reason for attending)*	
Mrs Irene Allan	Co-ordinator	
Mrs Carol Irvine	Ethics Co-ordinator	
Dr Rachel Venables	Scientific Officer (for Observation as part of Quality Control)	

Research Protocol

Title
Metabolomics and Inflammatory Bowel Disease

Background
Inflammatory bowel disease (IBD) is a common, chronic gastrointestinal disease comprised of two major subtypes, Crohn's disease (CD) and ulcerative colitis (UC). Both disorders have complex multifactorial aetiologies involving an inadequately defined relationship between microbial population insult, genetic predisposition and altered intestinal barrier permeability[1]. CD is characterised by patchy transmural granulomatous inflammation, affecting any part of the gastrointestinal tract, whereas UC is characterised by diffuse superficial mucosal inflammation, limited to the colon and rectum without granulomata.

Recent data suggests that the prevalence of IBD in the UK has exponentially increased to the current estimates of around 400 per 100,000[2]. Hospital statistics from England and Scotland also show that during the last two decades there is a significant increase in the hospitalisation rates for both diseases outlining the enormous economic impact to health services[3]. The age distribution is bimodal with a prominent first peak occurring in younger patients with CD and a more prominent second peak occurring in older patients with UC[iii]. This distribution has long ranging implications for the viable younger workforce and also the older dependant population. More importantly, the incidence rate of juvenile onset CD has risen by nearly 30% in Scotland over the last twenty years, which translates into a longer period of monitored healthcare for affected individuals[4].

Diagnostic and monitoring tools for IBD are currently inadequate. Numerous faecal biomarkers including calprotectin and lactoferrin[5,6] and serological biomarkers (ASCA, pANCA, anti-OmpC, anti-Cbir anti-I2 antibodies and anti-glycan antibodies) have been widely investigated within the last decade, but despite some promising studies, confirmation of their clinical validity and utility is awaited[7].

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[1] Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. *Nature* 2007;448(7152):427-434.
[2] Stone MA, Mayberry JF, Baker R. Prevalence and management of inflammatory bowel disease: a cross sectional study from central England. Eur J Gastroenterol Hepatol 2003, 15:12751280.
[3] Sonnenberg A. Temporal changes in the age distribution of inflammatory bowel disease hospitalization: data from England and Scotland. Eur J Gastroenterol Hepatol, 22:95101.
[4] Armitage E, Drummond HE, Wilson DC, Ghosh S. Increasing incidence of both juvenile onset Crohn's disease and ulcerative colitis in Scotland. Eur J Gastroenterol Hepatol 2001, 13:14391447.
[5] Konikoff MR, Denson LA. Role of fecal calprotectin as a biomarker of intestinal inflammation in inflammatory bowel disease. *Inflammatory Bowel Diseases* 2006;12(6):524-534.
[6] Schoepfer AM, Trummler M, Seeholzer P, Criblez DH, Seibold F. Accuracy of four fecal assays in the diagnosis of colitis. *Diseases of the Colon & Rectum* 2007;50(10):1697-1706.
[7] Seow CH, Stempak JM, Xu W, et al. Novel Anti-Glycan Antibodies Related to Inflammatory Bowel Disease Diagnosis and Phenotype. *American Journal of Gastroenterology* 2009;104(6):1426-1434.

Genetic studies have identified variants in over thirty genes, which alter the risk for CD[8,9] and genome wide association studies (GWAS) are now starting to unravel the genetic aetiology of UC[10,11,12,13]. These studies have provided exciting insights into the pathogenesis of IBD. However, each of these genetic variants only modestly increases the risk of disease and, either individually or in combination, they have limited clinical utility in aiding diagnosis of IBD, differentiating between UC and CD and predicting the natural history of disease. Moreover, environmental effects are largely missed using GWAS and this is very important in defining the phenotype of the disease (since phenotype = genotype + environment).

Metabolomics, and the associated metabonomics, are powerful scientific strategies which provide the investigation of low molecular weight (bio)chemicals (metabolites) present in the metabolome of a cell, tissue or organism[14,15]. Metabolomics focuses on the study of metabolism and the role of metabolites in regulatory processes including allosteric regulation and post-translational modifications.

We were the first group to investigate serum metabolomic profiles in patients with IBD. During discovery and validation studies we were able to define important metabolomic differences between CD, UC and matched controls. Univariate analysis demonstrated greater than 50 metabolites that varied in their relative concentration when comparing CD or UC versus control patients, whilst only 6 metabolites were shown to change when comparing CD versus UC. The greatest perturbation to metabolism was between healthy and disease subjects as would be expected, with a significantly lower level of differences observed between the two diseases. This may highlight similar pathophysiological mechanisms of both diseases with spatial differences in symptoms[16].

We now wish to interrogate these differences further by comparing metabolomic profiles of IBD patients during different phases of their disease. Metabolomic profiles will change with disease activity, anatomical distribution and therapy. Studying these changes will enable us to gain a greater understanding of the pathogenesis of

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[8] Hugot JP, Chamaillard M, Zouali H, et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001;411(6837):599-603.

[9] Ogura Y, Bonen DK, Inohara N, et al. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 2001;411(6837):603-606.

[10] Barrett JC, Lee JC, Lees CW, et al. Genome-wide association study of ulcerative colitis identifies three new susceptibility loci, including the HNF4A region. *Nature Genetics* 2009;41(12):1330-U99.

[11] Franke A, Balschun T, Sina C, et al. Genome-wide association study for ulcerative colitis identifies risk loci at 7q22 and 22q13 (IL17REL). *Nature Genetics* 2010:in press.

[12] McGovern DP, Gardet A, Törkvist L, et al. Genome-wide association identifies multiple ulcerative colitis susceptibility loci. *Nature Genetics* 2010:in press.

[13] Silverberg MS, Cho JH, Rioux JD, et al. Ulcerative colitis-risk loci on chromosomes 1p36 and 12q15 found by genome-wide association study. *Nature Genetics* 2009;41(2):216-220.

[14] Goodacre R, Vaidyanathan S, Dunn WB, Harrigan GG, Kell DB. Metabolomics by numbers: acquiring and understanding global metabolite data. *Trends in Biotechnology* 2004;22(5):245-252.

[15] Nicholson JK, Wilson ID. Understanding 'global' systems biology: Metabonomics and the continuum of metabolism. *Nature Reviews Drug Discovery* 2003;2(8):668-676.

[16] Johnston C, Dunn W, Broadhurst D, Brown M, Makin A, Campbell S, et al. Serum metabolite profiles differentiate Crohn's disease from ulcerative colitis and from healthy controls. Br J Surg 2010;97:S23.

the disease and may identify specific novel metabolic markers of disease identity and activity.

Principle Research Objectives

1. Define the relationship between metabolomic profiles and disease activity.
2. Examine how metabolomic profiles differ after surgery for inflammatory bowel disease.
3. Establish how metabolomic profiles of drug naïve IBD patients differ and how these alter after the introduction of medical therapy.

Methodology

Patients with inflammatory bowel disease in the Highlands and Islands of Scotland will be approached to take part in the study. We aim to recruit 40 drug naïve patients, 30 patients having surgery for IBD and 40 IBD patients receiving biological therapy. In addition 40 patients with active IBD will be recruited for serial sample collection (samples collected every 3 months for 1 year).

To recruit patients in the serial sampling group IBD patients will be sent metabolomics and IBD patient letter of invitation, version 3, and metabolomics participant information sheet 1 (IBD quarterly template), version 2, with routine clinic or endoscopy appointment letters. During their appointment they will be initially approached by the clinician in charge of their care regarding the study. Should they wish to gain more information they will then be seen by a member of the research team.

It will be ensured that potential participants have a copy of metabolomics participant information sheet 1 (IBD quarterly template), version 2. Participants will read the information sheet and be given the opportunity to discuss the study with a member of the research team and ask any questions about the study. Patients will be given the length of time they deem necessary to decide whether or not they wish to participate. Therefore, patients who are ready to make a decision to take part in the outpatient clinic (having being provided with and read the patient information sheet and discussed the study with their clinician and the research fellow and/or research nurse) can do so at that time. Alternatively patients can be followed up by a telephone call from the local recruitment officer/nurse to provide further information, if required, and consented to the study, if they are willing, when they next attend for a hospital appointment.

After informed consent the patients will be phenotyped and will have disease scoring carried out by means of a short interview with one of the research team. Blood (20ml) and urine (20ml) will be collected in the fasting state (>6 hours). In order to follow the relationship between disease activity and the metabolome (serum and urine), serial samples will be taken from consenting patients at three monthly intervals for a maximum duration of twelve months. The associated details of disease and drug activity will also be collated at these times. The timing of these samples will be correlated as far as possible with routine hospital appointments.

We will recruit drug naïve patients from clinic, the endoscopy unit, and from the inpatient wards. They will initially be approached by the clinician in charge of their care. If they wish to consider the study they will be given participant information sheet 1 (IBD quarterly template), version 2, and will meet with a member of the research team. They will have the opportunity to read the information sheet and ask

any questions regarding the study. They will be given the length of time they deem necessary to decide whether or not they wish to participate. If they are an out patient they may take the information home to consider it and will be followed up and consented, if willing, as per the serial sampling group. If they are an inpatient they will be given the length of time they need to decide whether or not to participate in the study. This is likely to be over 24 hours in most cases, however if a patient is willing to give informed consent having read the information and discussed the study with their own clinician and the research team consent may be taken in less than 24 hours. The reason for doing this would be to correlate fasting samples with routine morning blood samples that are taken after a night time fast.

To enable us to examine the change in the metabolome after surgery we will recruit patients undergoing surgery for IBD from clinic and endoscopy as well as from the inpatient wards. Patients will initially be approached by the clinician in charge of their care. They will be given metabolomics participant information sheet 1 (IBD surgery template), version 2, and then will meet with the research team if they are agreeable. They will have the opportunity to ask and questions and discuss the study. If they are an outpatient they will be able to go home with the information sheet and if they wish to participate written informed consent will be taken on attendance at the pre-assessment clinic or on the inpatient ward when they attend for surgery.
Those patients who are inpatients be given the length of time they need to decide whether or not to participate in the study. This is likely to be over 24 hours in most cases, however if a patient is willing to give informed consent having read the information and discussed the study with their own clinician and the research team consent may be taken in less than 24 hours. The reason for this would be if they required an urgent surgical procedure within 24 hours.
Patients will be phenotyped, have disease severity scoring carried out, and samples of blood (20ml) and urine (20ml) will be taken immediately prior to surgery as the patient will already be in the fasting state. Post surgery samples (20ml blood and 20ml urine) will be taken at a routine surgical clinic approximately 8 weeks post operatively. Disease severity scoring will be carried out at this time.

Patients who will receive biological therapy will be recruited from clinic, the endoscopy unit or the inpatient wards. They will initially be approached by the clinician in charge of their care. They will be given metabolomics participant information sheet 1 (IBD biological template), version 2, and then will meet with the research team if they are agreeable. They will have the opportunity to ask and questions and discuss the study. If they are an outpatient they will be able to go home with the information sheet and if they wish to participate written informed consent will be taken on attendance at the inpatient ward when they attend for therapy.
Those patients who are inpatients be given the length of time they need to decide whether or not to participate in the study. This is likely to be over 24 hours in most cases, however if a patient is willing to give informed consent having read the information and discussed the study with their own clinician and the research team consent may be taken in less than 24 hours. The reason for this would be if they required an urgent biological therapy within 24 hours.
Patients will be phenotyped, have disease severity scoring carried out, and samples of blood (20ml) and urine (20ml) will be taken immediately prior to biological therapy. Post therapy samples (20ml blood and 20ml urine) will be taken 2 weeks after treatment, when the patient attends for their next dose. Disease severity scoring will be carried out at this time.

It is potentially possible for one patient to be suitable for more than one of the groups. For example they may be in the serial sampling group but require biological therapy or surgery during the year of their participation in the study. Should this be the case they would be approached by their own clinician and shown the appropriate participant information sheet. If they wished further information they would meet with a member of the research team and have the opportunity to ask any questions about the study. They would then be consented and have their samples taken as previously described.

Suitably matched control subjects will be recruited from patients attending for elective, non-colorectal procedures with no personal or family history of colorectal disease. They will initially be approached at clinic by the clinician in charge of their care. If they are agreeable they will be given metabolomics participant information sheet 2 (control template), version 3. They will be given the length of time they deem necessary to decide whether or not they wish to participate. Therefore, patients who are ready to make a decision to take part in the outpatient clinic (having been provided with and read the patient information sheet and discussed the study with their clinician and the research fellow and/or research nurse) can do so at that time. Alternatively patients can be followed up by a telephone call from the local recruitment officer/nurse to provide further information, if required, and consented to the study, if they are willing, when they next attend for their surgical procedure. They will be phenotyped and will have samples of blood (20ml) and urine (20ml) taken in the fasting state. Repeat samples are not required in the control group.

During phenotyping the following patient specific and disease specific information will be collated during an interview with a member of the research team and by review of the medical notes:

Patient Specific Information

- Date of birth
- Place of birth
- Current postcode
- Sex
- Ethnicity
- Age at diagnosis
- Height / weight / BMI
- Social history
- Past medical history
- Drug history
- Past surgical history
- Family history

Disease Specific Information

- Diagnosis (Crohn's Disease / Ulcerative Colitis / Indeterminate Colitis)
    - Clinical / radiological / endoscopic / pathological
- Disease location
- Disease behaviour (nonstricturing nonpenetrating / fistulating / stenosing)

Disease Severity Scoring

- Harvey-Bradshaw Index (Crohn's Disease)

    - General wellbeing, abdominal pain, number of liquid stools / day, abdominal mass, complications of CD
- Simple Clinical Colitis Activity Index (Ulcerative Colitis)
    - Bowel frequency (day / night), urgency of defaecation, blood in the stool, general wellbeing, extracolonic features

Consent forms will be stored in a locked filing cabinet in a locked office in the Centre for Health Science, Raigmore Hospital, Inverness.
All data will be stored on a secure database on an NHS computer. This computer is in the Centre for Health Science, Raigmore Hospital, Inverness, in an office that an access card is required to gain entry.

During informed consent, the participants will be asked to consent to their GP being informed of their involvement in the study. All of the GPs within Highland will be informed of the study by means of metabolomics and IBD GP letter and information sheet 1, version 2. Should one of their patients with IBD enrol in the study the GP will be informed by means of metabolomics and IBD GP letter and information sheet 2, version 2. If one of their patients is enrolled as a control the GP will be informed by means of metabolomics and IBD GP letter and information sheet 3, version 1.

All blood samples will be allowed to clot for <120 minutes on ice at 4℃ and serum samples will be obtained by centrifuging the samples at 2500g for 15 minutes at 4℃. Urine will be put on ice for <120 minutes. Sam ples will then be stored in linked anonymised form in a -80℃ freezer in the Centre fo r Health Science, Raigmore Hospital, Inverness.

Samples of serum and urine will be transported to Manchester in liquid nitrogen using a specialist laboratory courier service. All metabolomic studies will be performed at the Manchester Integrative Biocentre under the supervision of Professor Roy Goodacre and Dr Warwick Dunn. Serum samples will be deproteinised in methanol and analysed using UPLC-MS (Waters AQUITY UPLC coupled to a ThermoFisher LTQ-Orbitrap MS). Urine samples will be processed with urease to remove urea and analysed using GC-MS (Agilent 6890N GC coupled to a Leco Pegasus III MS). Quality control samples will be intermittently analysed to provide quality assurance of the data acquired. Raw data will be processed using the XCMS software package (UPLC-MS) and Leco ChromaTof software package (GC-MS). Data analysis including univariate and multivariate statistics will combine analytical and clinical data to enable a greater understanding of the interactions between clinical phenotype and metabolome related to objectives set.

Research Environment
A key collaboration has been established with Professor Roy Goodacre and Dr Warwick Dunn (University of Manchester) which was started whilst Mr Angus Watson worked at Manchester Royal Infirmary. Clinical metabolomics research in Manchester has an international reputation of excellence. The Manchester Centre for Integrative Systems Biology (www.mcisb.org), of which Professor Goodacre is a PI, provides the resources and expertise to perform this type of research.
There are over 600 patients with inflammatory bowel disease in the Highlands & Islands of Scotland. The majority of specialist gastroenterology and surgical services are delivered at Raigmore Hospital in Inverness. Research activity is based in the Centre for Health Science (www.centreforhealthscience.com) and the Highland Clinical Research Facility.

Contacts

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Name: << DATE >>

Address:

Title of the Study: **Metabolomics and Inflammatory Bowel Disease (IBD)**

Dear Sir / Madam

You have been scheduled for an outpatient appointment in the near future in Raigmore Hospital. I would like to draw your attention to an important study that is ongoing in Raigmore Hospital. This study involves taking samples of blood and urine from adults with IBD at three monthly intervals for one year, at the same time as assessing disease activity by means of a short interview. We will measure biochemicals in the samples and correlate them with disease activity. We hope that this will allow us to better diagnose and treat IBD in the future.

I have included an information sheet with this letter for you to read.

A member of the research team will be available to discuss this study with you in more detail and answer any questions you may have when you attend on the day of clinic.

With kind regards

Yours sincerely,

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Metabolomics Participant Information Sheet
IBD Surgery Version

Study title: Metabolomics and inflammatory bowel disease

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you wish to take part. Thank you for reading this.

What is the purpose of the study?
Crohn's disease and ulcerative colitis are chronic inflammatory bowel diseases (IBD) of significant importance to both individual patients and the wider health economy. We are still uncertain as to what actually causes these diseases. We urgently need to develop techniques that will help us;
1. uncover the causes of IBD,
2. identify chemical markers of disease activity,
3. measure responses to treatments.

Metabolomics is a powerful new scientific technique that measures huge numbers of biochemicals. In pilot studies biochemicals have been identified which help tell apart IBD patients from normal subjects and to a lesser extent can differentiate between Crohn's disease and ulcerative colitis. We now wish to build on these new findings and over the next 2 years we will collect samples of blood and urine from patients undergoing surgery, those having biological therapy and at 3 monthly intervals for those on medical treatment to analyse biochemical markers.

Why have I been chosen?
All patients with IBD having surgery in the Highlands and Islands will be approached to participate.
We will also be recruiting control patients.

Do I have to take part?
No. It is up to you to decide whether to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?

A member of the research team will discuss the study with you. You will be given this information sheet to keep, and will be able to ask any questions that you have regarding the study. If you are an out patient you will be able to go home and consider whether or not you wish to be involved. If you are an inpatient you will be given as much time as you require deciding whether or not you wish to participate. If you agree to take part you will be asked to sign a consent form and complete an interview with the researcher to allow us to gather demographic information and assess the current activity of your disease.
We will ask for samples of 20ml (four teaspoons) of blood and 20ml of urine immediately prior to your surgical procedure, and approximately 8 weeks afterwards during a routine clinic visit. At this time we will again assess the activity of your disease by a short interview.
All samples must be taken in a fasting state, which means you cannot eat or drink for 6 hours prior to the sample being taken.

What are the possible disadvantages and risks of taking part?
A blood sample is required but often this is part of the normal care you would receive and most people tolerate it very well.

What are the possible benefits of taking part?
There will be no clinical benefit to you but from this research we hope to be able to discover new biochemical markers that may help us to diagnose and treat IBD in the future.

What happens when the research study stops?
Once the study is complete your data will be stored for 3 years, pending review. Any remaining samples will be stored for potential use in future studies. Any future work using stored tissue samples will be subject to approval from the local ethics committee.

Will my taking part in this study be kept confidential?
All the information collected is confidential. On agreeing to donate samples for research, your name, along with other personal information, including date of birth and hospital number, will be entered into a file alongside a unique code number. The research team will store this file of personal information and codes securely on an NHS computer. All samples collected will be stored and labelled with the code number only but no personal details. Thus, all laboratory work will involve the use of the code number only and will have no direct link to personal details, ensuring anonymity.

Will my GP be informed?
All GPs in Highland have been sent an information pack with the details of the study. We will ask for your permission to inform your own GP of your involvement.

What will happen to the results of the research study?
The study will form the basis of an MD thesis, and results are likely to be published in a medical journal and presented at scientific / medical meetings. You would never be identified during any of these processes.

Who is organising and funding the research?
NHS Highland
The doctor conducting the research is not being paid for conducting this study, but continues to work for NHS Highland in the Department of General Surgery.

Who has reviewed the study?
This study has been reviewed by the North of Scotland Research Ethics Committee.

How to complain
If you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal NHS complaints mechanisms are available to you.

What next?
If you are interested in participating in the study please complete the last page of this information sheet and hand it in to the reception staff at the clinic. A member of the research team can then meet with you to discuss your participation in the study in more detail.

Thank you very much for taking the time to read this information sheet and considering taking part in this study.

Contact for Further Information

Miss Diane Hildebrand
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Consultant Surgeon
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I am interested in finding out more about the research study "Metabolomics and inflammatory bowel disease" and would like to meet with a member of the research team.

Name _____

Signature _____

Date ____/____/____

Metabolomics Participant Information Sheet
IBD Quarterly Version

Study title: Metabolomics and inflammatory bowel disease

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you wish to take part. Thank you for reading this.

What is the purpose of the study?
Crohn's disease and ulcerative colitis are chronic inflammatory bowel diseases (IBD) of significant importance to both individual patients and the wider health economy. We are still uncertain as to what actually causes these diseases. We urgently need to develop techniques that will help us;
1. uncover the causes of IBD,
2. identify chemical markers of disease activity,
3. measure responses to treatments.

Metabolomics is a powerful new scientific technique that measures huge numbers of biochemicals. In pilot studies biochemicals have been identified which help tell apart IBD patients from normal subjects and to a lesser extent can differentiate between Crohn's disease and ulcerative colitis. We now wish to build on these new findings and over the next 2 years we will collect samples of blood and urine from patients from patients undergoing surgery, those having biological therapy and at 3 monthly intervals for those on medical treatment to analyse biochemical markers.

Why have I been chosen?
All patients with IBD in the Highlands and Islands will be approached to participate. We will also be recruiting control patients.

Do I have to take part?
No. It is up to you to decide whether to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?

A member of the research team will discuss the study with you. You will be given this information sheet to keep, and will be able to ask any questions that you have regarding the study. If you are an out patient you will be able to go home and consider whether or not you wish to be involved. If you are an inpatient you will be given as much time as you require deciding whether or not you wish to participate. If you agree to take part you will be asked to sign a consent form and complete an interview with the researcher to allow us to gather demographic information and assess the current activity of your disease.
We will ask for samples of 20ml (four teaspoons) of blood and 20ml of urine at 3 monthly intervals for 1 year, as well as a short interview to assess the activity of your disease.
All samples must be taken in a fasting state, which means you cannot eat or drink for 6 hours prior to the sample being taken.
As far as possible we aim to correlate samples with hospital visits and routine blood tests.

What are the possible disadvantages and risks of taking part?
We require a commitment to 3 monthly samples of blood and urine.

What are the possible benefits of taking part?
There will be no clinical benefit to you but from this research we hope to be able to discover new biochemical markers that may help us to diagnose and treat IBD in the future.

What happens when the research study stops?
Once the study is complete your data will be stored for 3 years, pending review. Any remaining samples will be stored for potential use in future studies. Any future work using stored tissue samples will be subject to approval from the local ethics committee.

Will my taking part in this study be kept confidential?
All the information collected is confidential. On agreeing to donate samples for research, your name, along with other personal information, including date of birth and hospital number, will be entered into a file alongside a unique code number. The research team will store this file of personal information and codes securely on an NHS computer. All samples collected will be stored and labelled with the code number only but no personal details. Thus, all laboratory work will involve the use of the code number only and will have no direct link to personal details, ensuring anonymity.

Will my GP be informed?
All GPs in Highland have been sent an information pack with the details of the study. We will ask for your permission to inform your own GP of your involvement.

What will happen to the results of the research study?
The study will form the basis of an MD thesis, and results are likely to be published in a medical journal and presented at scientific / medical meetings. You would never be identified during any of these processes.

Who is organising and funding the research?
NHS Highland
The doctor conducting the research is not being paid for conducting this study, but continues to work for NHS Highland in the Department of General Surgery.

Who has reviewed the study?
This study has been reviewed by the North of Scotland Research Ethics Committee.

How to complain
If you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal NHS complaints mechanisms are available to you.

What next?
If you are interested in participating in the study please complete the last page of this information sheet and hand it in to the reception staff at the clinic. A member of the research team can then meet with you to discuss your participation in the study in more detail.

Thank you very much for taking the time to read this information sheet and considering taking part in this study.

Contact for Further Information

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I am interested in finding out more about the research study "Metabolomics and inflammatory bowel disease" and would like to meet with a member of the research team.

Name _____

Signature _____

Date ____/____/____

Metabolomics Participant Information Sheet
IBD Biological Version

Study title: Metabolomics and inflammatory bowel disease

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you wish to take part. Thank you for reading this.

What is the purpose of the study?
Crohn's disease and ulcerative colitis are chronic inflammatory bowel diseases (IBD) of significant importance to both individual patients and the wider health economy. We are still uncertain as to what actually causes these diseases. We urgently need to develop techniques that will help us;
1. uncover the causes of IBD,
2. identify chemical markers of disease activity,
3. measure responses to treatments.

Metabolomics is a powerful new scientific technique that measures huge numbers of biochemicals. In pilot studies biochemicals have been identified which help tell apart IBD patients from normal subjects and to a lesser extent can differentiate between Crohn's disease and ulcerative colitis. We now wish to build on these new findings and over the next 2 years we will collect samples of blood and urine from patients undergoing surgery, those having biological therapy and at 3 monthly intervals for those on medical treatment to analyse biochemical markers.

Why have I been chosen?
All patients with IBD having biological therapy in the Highlands and Islands will be approached to participate.
We will also be recruiting control patients.

Do I have to take part?
No. It is up to you to decide whether to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?
A member of the research team will discuss the study with you. You will be given this information sheet to keep, and will be able to ask any questions that you have regarding the study. If you are an out patient you will be able to go home and consider whether or not you wish to be involved. If you are an inpatient you will be given as much time as you require deciding whether or not you wish to participate. If you agree to take part you will be asked to sign a consent form and complete an interview with the researcher to allow us to gather demographic information and assess the current activity of your disease. We will ask for samples of 20ml (four teaspoons) of blood and 20ml of urine immediately prior to your biological therapy, and approximately 2 weeks afterwards during a routine hospital visit. At this time we will again assess the activity of your disease by a short interview.
All samples must be taken in a fasting state, which means you cannot eat or drink for 6 hours prior to the sample being taken.

What are the possible disadvantages and risks of taking part?
A blood sample is required but often this is part of the normal care you would receive and most people tolerate it very well.

What are the possible benefits of taking part?
There will be no clinical benefit to you but from this research we hope to be able to discover new biochemical markers that may help us to diagnose and treat IBD in the future.

What happens when the research study stops?
Once the study is complete your data will be stored for 3 years, pending review. Any remaining samples will be stored for potential use in future studies. Any future work using stored tissue samples will be subject to approval from the local ethics committee.

Will my taking part in this study be kept confidential?
All the information collected is confidential. On agreeing to donate samples for research, your name, along with other personal information, including date of birth and hospital number, will be entered into a file alongside a unique code number. The research team will store this file of personal information and codes securely on an NHS computer. All samples collected will be stored and labelled with the code number only but no personal details. Thus, all laboratory work will involve the use of the code number only and will have no direct link to personal details, ensuring anonymity.

Will my GP be informed?
All GPs in Highland have been sent an information pack with the details of the study. We will ask for your permission to inform your own GP of your involvement.

What will happen to the results of the research study?
The study will form the basis of an MD thesis, and results are likely to be published in a medical journal and presented at scientific / medical meetings. You would never be identified during any of these processes.

Who is organising and funding the research?
NHS Highland
The doctor conducting the research is not being paid for conducting this study, but continues to work for NHS Highland in the Department of General Surgery.

Who has reviewed the study?
This study has been reviewed by the North of Scotland Research Ethics Committee.

How to complain
If you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal NHS complaints mechanisms are available to you.

What next?
If you are interested in participating in the study please complete the last page of this information sheet and hand it in to the reception staff at the clinic. A member of the research team can then meet with you to discuss your participation in the study in more detail.

Thank you very much for taking the time to read this information sheet and considering taking part in this study.

Contact for Further Information

Miss Diane Hildebrand
Clinical Research Fellow
Centre for Health Science,
Raigmore Hospital,
Inverness
IV2 3UJ
Email: dianehildebrand@nhs.net
Tel: 01463 279 575

Professor Angus Watson
Chief Investigator
Consultant Surgeon
Director of R&D
Raigmore Hospital
Inverness, IV2 3UJ
angus.watson@nhs.net
01463 705 414

I am interested in finding out more about the research study "Metabolomics and inflammatory bowel disease" and would like to meet with a member of the research team.

Name _____

Signature _____

Date ____/____/____

Metabolomics Participant Information Sheet
Control Version

Study title: Metabolomics and inflammatory bowel disease

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you wish to take part. Thank you for reading this.

What is the purpose of the study?
Crohn's disease and ulcerative colitis are chronic inflammatory bowel diseases (IBD) of significant importance to both individual patients and the wider health economy. We are still uncertain as to what actually causes these diseases. We urgently need to develop techniques that will help us;
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Metabolomics is a powerful new scientific technique that measures huge numbers of biochemicals. In pilot studies biochemicals have been identified which help tell apart IBD patients from normal subjects and to a lesser extent can differentiate between Crohn's disease and ulcerative colitis. We now wish to build on these new findings and over the next 2 years we will collect samples of blood and urine from patients at 3 monthly intervals to analyse biochemical markers.

Why have I been chosen?
All patients with IBD in the Highlands and Islands will be approached to participate. We will also be recruiting control patients from out patient clinics. These patients will be attending for elective non-colorectal procedures and must have no personal or family history of colorectal disease.

Do I have to take part?
No. It is up to you to decide whether to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?

At the out patient clinic a member of the research team will discuss the study with you. You will be given this information sheet to keep, and will be able to ask any questions that you have regarding the study. You will be able to go home and consider whether or not you wish to be involved.
On the day that you attend for your elective surgical procedure you will be seen by a member of the research team. You will be able to ask any further questions and if you wish to participate you will be asked to sign a consent form and then to complete an interview with the researcher to allow us to gather demographic information.
We will take a 20ml (four teaspoons) blood sample and a 20ml urine sample from you.
The samples must be taken in a fasting state, which means you cannot eat of drink anything for 6 hours prior to the sample being taken.

What are the possible disadvantages and risks of taking part?
A blood sample is required but often this is part of the normal care you would receive and most people tolerate it very well.

What are the possible benefits of taking part?
There will be no clinical benefit to you but from this research we hope to be able to discover new biochemical markers that may help us to diagnose and treat IBD in the future.

What happens when the research study stops?
Once the study is complete your data will be stored for 3 years, pending review. Any remaining samples will be stored for potential use in future studies. Any future work using stored tissue samples will be subject to approval from the local ethics committee.

Will my taking part in this study be kept confidential?
All the information collected is confidential. On agreeing to donate samples for research, your name, along with other personal information, including date of birth and hospital number, will be entered into a file alongside a unique code number. The research team will store this file of personal information and codes securely on an NHS computer. All samples collected will be stored and labelled with the code number only but no personal details. Thus, all laboratory work will involve the use of the code number only and will have no direct link to personal details, ensuring anonymity.

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The study will form the basis of an MD thesis, and results are likely to be published in a medical journal and presented at scientific / medical meetings. You would never be identified during any of these processes.

Who is organising and funding the research?
NHS Highland.
The doctor conducting the research is not being paid for conducting this study, but continues to work for NHS Highland in the Department of General Surgery.

Who has reviewed the study?
This study has been reviewed by the North of Scotland Research Ethics Committee.

How to complain

If you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal NHS complaints mechanisms are available to you.

What next?
If you are interested in participating in the study please complete the last page of this information sheet and hand it in to the reception staff at the clinic. A member of the research team can then meet with you to discuss your participation in the study in more detail.

Thank you very much for taking the time to read this information sheet and considering taking part in this study.

Contact for Further Information

Miss Diane Hildebrand
Clinical Research Fellow
Centre for Health Science,
Raigmore Hospital,
Inverness
IV2 3UJ
Email: dianehildebrand@nhs.net
Tel: 01463 279 575

Professor Angus Watson
Chief Investigator
Consultant Surgeon
Director of R&D
Raigmore Hospital
Inverness, IV2 3UJ
angus.watson@nhs.net
01463 705 414

I am interested in finding out more about the research study "Metabolomics and inflammatory bowel disease" and would like to meet with a member of the research team.

Name _____

Signature _____

Date ____/____/____

Name: << DATE >>

Address:

Title of the Study: **Metabolomics and Inflammatory Bowel Disease (IBD)**

Dear Sir / Madam

You have been scheduled for an outpatient appointment in the near future in Raigmore Hospital. I would like to draw your attention to an important study that is ongoing in Raigmore Hospital. This study involves taking samples of blood and urine from adults with IBD at three monthly intervals for one year, at the same time as assessing disease activity by means of a short interview. We will measure biochemicals in the samples and correlate them with disease activity. We hope that this will allow us to better diagnose and treat IBD in the future.

I have included an information sheet with this letter for you to read.

A member of the research team will be available to discuss this study with you in more detail and answer any questions you may have when you attend on the day of clinic.

With kind regards

Yours sincerely,

Miss Diane Hildebrand
Clinical Research Fellow
Centre for Health Science
Raigmore Hospital
Old Perth Road
Inverness
IV2 3UJ

Email: dianehildebrand@nhs.net
Tel: 01463 279 575

Professor Angus Watson
Chief Investigator
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Raigmore Hospital
Old Perth Road
Inverness
IV2 3UJ
angus.watson@nhs.net
01463 705 414

8.1.8 Metabolomics and IBD Control Letter of Invitation, 15/06/11

<< DATE >>

Title of the Study: **Metabolomics and Inflammatory Bowel Disease (IBD)**

Dear Sir / Madam

You have been scheduled for an elective operation in the near future in Raigmore Hospital. I would like to draw your attention to an important study that is ongoing in Raigmore Hospital. This study involves taking samples of blood and urine from adults with IBD. We will measure biochemicals in the samples and correlate them with disease activity. We hope that this will allow us to better diagnose and treat IBD in the future.

We are currently recruiting healthy control patients to compare to the patients with IBD. This would involve giving a sample of blood and urine

I have included an information sheet with this letter for you to read.

Should you wish a member of the research team will be available to discuss this study with you in more detail and answer any questions you may have when you attend on the day of your procedure.

With kind regards

Yours sincerely,

Miss Diane Hildebrand
Clinical Research Fellow
Centre for Health Science
Raigmore Hospital
Old Perth Road
Inverness
IV2 3UJ

Email: dianehildebrand@nhs.net
Tel: 01463 279 575

Professor Angus Watson
Chief Investigator
Consultant Surgeon /
Director of R&D
Raigmore Hospital
Old Perth Road
Inverness
IV2 3UJ
angus.watson@nhs.net
01463 705 414

<< DATE >>

Dear Consultant,

Metabolomics and Inflammatory Bowel Disease

I am writing to draw your attention to the above study that will be ongoing in Raigmore Hospital for the next two years, and will form the basis of my MD thesis.

This is a study into metabolomics (biomarkers) in inflammatory bowel disease. Our aims are to:

1. Define the relationship between metabolomic profiles and disease activity.
2. Examine how metabolomic profiles differ after surgery for inflammatory bowel disease.
3. Establish how metabolomic profiles of drug naïve IBD patients differ and how these alter after the introduction of medical therapy.

As part of the study we require to enrol healthy controls from whom to obtain fasting samples of blood and urine. We will be recruiting from outpatient clinics. We aim to enrol patients who require elective, non-colorectal procedures with no personal or family history of colorectal disease.

All aspects of the study including informed consent, and the collection of samples, which will be taken on the day of surgery, will be dealt with by the research team. At no point will this intrude on the care that you are providing.

Please see overleaf for further information. Should you wish to discuss any aspect of our study or if you have any concerns regarding the potential recruitment of your patients please do not hesitate to contact me.

Yours sincerely,

Miss Diane Hildebrand
Clinical Research Fellow
Centre for Health Science
Raigmore Hospital
Old Perth Road
Inverness
IV2 3UJ

Email: dianehildebrand@nhs.net
Tel: 01463 279 575

Professor Angus Watson
Chief Investigator
Consultant Surgeon /
Director of R&D
Raigmore Hospital
Old Perth Road
Inverness
IV2 3UJ

angus.watson@nhs.net
01463 705 414

Metabolomics and Inflammatory Bowel Disease
Consultant Information Sheet

Title of project: Metabolomics and Inflammatory Bowel Disease

Background

Crohn's disease and ulcerative colitis are two types of inflammatory bowel disorders (IBD) of unknown aetiology that result in significant morbidity and health expenditure. Recent data suggests that the prevalence of IBD in the UK has exponentially increased to the current estimates of around 400 per 100,000. Hospital statistics from England and Scotland also show that during the last two decades there is a significant increase in the hospitalisation rates for both diseases outlining the enormous economic impact to health services. The age distribution is bimodal with a prominent first peak occurring in younger patients with Crohn's disease and a more prominent second peak occurring in older patients with ulcerative colitis. This distribution has long ranging implications for the viable younger workforce and also the older dependant population. More importantly, the incidence rate of juvenile onset Crohn's disease has risen by nearly 30% in Scotland over the last twenty years, which translates into a longer period of monitored healthcare for affected individuals.

Diagnostic and monitoring tools for IBD are currently inadequate. Numerous faecal biomarkers have been widely investigated within the last decade, but despite some promising studies, confirmation of their clinical validity and utility is awaited.

Metabolomics, and the associated metabonomics, are powerful scientific strategies which provide the investigation of low molecular weight (bio)chemicals (metabolites) present in the metabolome of a cell, tissue or organism.

Professor Watson was involved in the first group to investigate serum metabolomic profiles in patients with IBD. During discovery and validation studies it was possible to define important metabolomic differences between Crohn's disease, ulcerative colitis and matched controls.

We now wish to interrogate these differences further by comparing metabolomic profiles of IBD patients during different phases of their disease. Metabolomic profiles will change with disease activity, anatomical distribution and therapy. Studying these changes will enable us to gain a greater understanding of the pathogenesis of the disease and may identify specific novel metabolic markers of disease identity and activity.

Brief outline of the study

Ethical approval has been granted for this study.
Patients with inflammatory bowel disease in the Highlands and Islands of Scotland will be approached to take part in the study. After informed consent the patients will be phenotyped and their data stored on a secure database. Disease activity will be recorded using the Harvey Bradshaw and Simple Clinical Colitis activity indices. Serum and urine will be collected in the fasting state (>6 hours).
To enable us to examine the change in the metabolome after surgery and the introduction of drug therapy, disease activity and phenotype will be recorded prior to the intervention. Urine and blood samples will also be collected at this time. This process will be repeated two months after surgery or after the start of medical therapy.
In order to follow the relationship between disease activity and the metabolome (serum and urine), serial samples will be taken from consenting patients at three monthly intervals for a maximum duration of 12 months. The associated details of disease and drug activity will also be collated at these times.

Control subjects will be recruited from patients attending for elective, non-colorectal procedures with no personal or family history of colorectal disease. Written consent will be taken, phenotype collected and a single sample of blood (20ml) and urine (20ml) taken in the fasting state.
All the data collected is confidential. Demographic data will be entered into a file alongside a unique code number. The research team will store this file of personal information and codes securely on an NHS computer. All samples collected will be stored and labelled with the code number only but no personal details. Thus, all laboratory work will involve the use of the code number only and will have no direct link to personal details, ensuring anonymity.
These samples will be transported to Manchester Integrative Biocentre where they will be analysed.

<< DATE >>

Dear Dr

Metabolomics and Inflammatory Bowel Disease

I am writing to make you aware of the above study that will be taking place in Raigmore Hospital.

This is a study into metabolomics (biomarkers) in inflammatory bowel disease. Our aims are to:

1. Define the relationship between metabolomic profiles and disease activity.
2. Examine how metabolomic profiles differ after surgery for inflammatory bowel disease.
3. Establish how metabolomic profiles of drug naïve IBD patients differ and how these alter after the introduction of medical therapy.

For further information please see overleaf.

If any of your patients agree to participate we will write and inform you. We should not normally need to obtain any information from you.

If you would like to discuss any aspect of our study, or require any further details, please do not hesitate to contact me at the email address given below.

Yours sincerely,

Miss Diane Hildebrand
Clinical Research Fellow
Centre for Health Science
Raigmore Hospital
Old Perth Road
Inverness
IV2 3UJ

Email: dianehildebrand@nhs.net
Tel: 01463 279 575

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Director of R&D
Raigmore Hospital
Old Perth Road
Inverness
IV2 3UJ

angus.watson@nhs.net
01463 705 414

Metabolomics and Inflammatory Bowel Disease
GP Information Sheet

Title of project: Metabolomics and Inflammatory Bowel Disease

Background

Crohn's disease and ulcerative colitis are two types of inflammatory bowel disorders (IBD) of unknown aetiology that result in significant morbidity and health expenditure. Recent data suggests that the prevalence of IBD in the UK has exponentially increased to the current estimates of around 400 per 100,000. Hospital statistics from England and Scotland also show that during the last two decades there is a significant increase in the hospitalisation rates for both diseases outlining the enormous economic impact to health services. The age distribution is bimodal with a prominent first peak occurring in younger patients with Crohn's disease and a more prominent second peak occurring in older patients with ulcerative colitis. This distribution has long ranging implications for the viable younger workforce and also the older dependant population. More importantly, the incidence rate of juvenile onset Crohn's disease has risen by nearly 30% in Scotland over the last twenty years, which translates into a longer period of monitored healthcare for affected individuals.

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We now wish to interrogate these differences further by comparing metabolomic profiles of IBD patients during different phases of their disease. Metabolomic profiles will change with disease activity, anatomical distribution and therapy. Studying these changes will enable us to gain a greater understanding of the pathogenesis of the disease and may identify specific novel metabolic markers of disease identity and activity.

Brief outline of the study

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To enable us to examine the change in the metabolome after surgery and the introduction of drug therapy, disease activity and phenotype will be recorded prior to the intervention. Urine and blood samples will also be collected at this time. This process will be repeated two months after surgery or after the start of medical therapy.
In order to follow the relationship between disease activity and the metabolome (serum and urine), serial samples will be taken from consenting patients at three monthly intervals for a maximum duration of 12 months. The associated details of disease and drug activity will also be collated at these times.

Control subjects will be recruited from patients attending for elective, non-colorectal procedures with no personal or family history of colorectal disease. Written consent will be taken, phenotype collected and a single sample of blood (20ml) and urine (20ml) taken in the fasting state.
All the data collected is confidential. Demographic data will be entered into a file alongside a unique code number. The research team will store this file of personal information and codes securely on an NHS computer. All samples collected will be stored and labelled with the code number only but no personal details. Thus, all laboratory work will involve the use of the code number only and will have no direct link to personal details, ensuring anonymity.
These samples will be transported to Manchester Integrative Biocentre where they will be analysed.

Dr GPFName GPSName
GPAddress1
GPAddress2
GPAddress3
GPAddress4
GPPostCode

<< DATE >>

Dear Dr GPSName

Metabolomics and Inflammatory Bowel Disease

The above study is ongoing at Raigmore Hospital. One of you patients, (insert patient details), who has IBD has agreed to participate.

This is a study into metabolomics (biomarkers) in inflammatory bowel disease. Our aims are to:

1. Define the relationship between metabolomic profiles and disease activity.
2. Examine how metabolomic profiles differ after surgery for inflammatory bowel disease.
3. Establish how metabolomic profiles of drug naïve IBD patients differ and how these alter after the introduction of medical therapy.

For further information please see overleaf.

Samples of blood and urine have been taken. We should not normally need to obtain any information from you. However, we would be grateful if you could email dianehildebrand@nhs.net if your patient changes address, or dies.

If you would like to discuss any aspect of our study, or require any further details, please do not hesitate to contact me at the above email address.

Yours sincerely,

Miss Diane Hildebrand
Clinical Research Fellow
Centre for Health Science
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Metabolomics and Inflammatory Bowel Disease
GP Information Sheet

Title of project: Metabolomics and Inflammatory Bowel Disease

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<< DATE >>

Dr GPFName GPSName
GPAddress1
GPAddress2
GPAddress3
GPAddress4
GPPostCode

Dear Dr GPSName

Metabolomics and Inflammatory Bowel Disease

The above study is ongoing at Raigmore Hospital. One of you patients (insert patient details) has agreed to participate as a control.

This is a study into metabolomics (biomarkers) in inflammatory bowel disease. Our aims are to:

1. Define the relationship between metabolomic profiles and disease activity.
2. Examine how metabolomic profiles differ after surgery for inflammatory bowel disease.
3. Establish how metabolomic profiles of drug naïve IBD patients differ and how these alter after the introduction of medical therapy.

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Metabolomics and Inflammatory Bowel Disease
GP Information Sheet

Title of project: Metabolomics and Inflammatory Bowel Disease

Background

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We now wish to interrogate these differences further by comparing metabolomic profiles of IBD patients during different phases of their disease. Metabolomic profiles will change with disease activity, anatomical distribution and therapy. Studying these changes will enable us to gain a greater understanding of the pathogenesis of the disease and may identify specific novel metabolic markers of disease identity and activity.

Brief outline of the study

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To enable us to examine the change in the metabolome after surgery and the introduction of drug therapy, disease activity and phenotype will be recorded prior to the intervention. Urine and blood samples will also be collected at this time. This process will be repeated two months after surgery or after the start of medical therapy.
In order to follow the relationship between disease activity and the metabolome (serum and urine), serial samples will be taken from consenting patients at three monthly intervals for a maximum duration of 12 months. The associated details of disease and drug activity will also be collated at these times.

Control subjects will be recruited from patients attending for elective, non-colorectal procedures with no personal or family history of colorectal disease. Written consent will be taken, phenotype collected and a single sample of blood (20ml) and urine (20ml) taken in the fasting state.
All the data collected is confidential. Demographic data will be entered into a file alongside a unique code number. The research team will store this file of personal information and codes securely on an NHS computer. All samples collected will be stored and labelled with the code number only but no personal details. Thus, all laboratory work will involve the use of the code number only and will have no direct link to personal details, ensuring anonymity.
These samples will be transported to Manchester Integrative Biocentre where they will be analysed.

Study Number: 11\AL\0238
Patient Identification Number for this trial:

PARTICIPANT CONSENT FORM

Title of Project: Metabolomics and Inflammatory Bowel Disease

Name of Researcher: Miss Diane Hildebrand, Clinical Research Fellow, Raigmore Hospital, Inverness
Email: dianehildebrand@nhs.net
Tel: 01463 279 575

Please initial box

1	I confirm that I have read and understand the metabolomics participant information sheet 1 (IBD ……template) version 2 dated 15/06/11 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
2	I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	
3	I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from NHS HIGHLAND, from regulatory authorities or from the NHS Board, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	
4	I agree to my GP being informed of my participation in the study.	
5	I agree to my samples being stored and used in future research studies where ethical approval has been granted.	
6	I agree to take part in the above study.	

_____	_____	_____
Name of Patient	Signature	Date
_____	_____	_____
Name of Person taking consent (if different from researcher)	Signature	Date
_____	_____	_____
Researcher	Signature	Date

1 for patient; 1 for researcher; 1 to be kept with hospital notes

Study Number: 11\AL\0238
Patient Identification Number for this trial:

PARTICIPANT CONSENT FORM

Title of Project: Metabolomics and Inflammatory Bowel Disease

Name of Researcher: Miss Diane Hildebrand, Clinical Research Fellow, Raigmore Hospital, Inverness
Email: dianehildebrand@nhs.net
Tel: 01463 279 575

Please initial box

1	I confirm that I have read and understand the metabolomics participant information sheet 2 (control template) version 3 dated 15/06/11 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
2	I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	
3	I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from NHS HIGHLAND, from regulatory authorities or from the NHS Board, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	
4	I agree to my GP being informed of my participation in the study.	
5	I agree to my samples being stored and used in future research studies where ethical approval has been granted.	
6	I agree to take part in the above study.	

_____	_____	_____
Name of Patient	Signature	Date
_____	_____	_____
Name of Person taking consent (if different from researcher)	Signature	Date
_____	_____	_____
Researcher	Signature	Date

1 for patient; 1 for researcher; 1 to be kept with hospital notes

8.2 Clinical Metadata 8.2.1 Healthy Controls										
Label	**Sex**	**Age**	**Nationality**	**Height (m)**	**Weight (kg)**	**BMI**	**Diet**	**Smoking**	**Alcohol excess**	**Inflammatory condition**
DRH061	M	46	White British	1.7	90	31.1	normal	never	no	no
DRH062	M	49	White British	1.86	90	26.0	normal	never	no	no
DRH064	M	47	White British	1.78	82.8	26.1	normal	never	no	no
DRH065	M	54	White British	1.77	82.5	26.3	normal	never	no	no
DRH069	M	64	White British	1.7	94	32.5	normal	ex	no	no
DRH070	M	44	White British	1.85	97.9	28.6	normal	ex	no	no
DRH073	M	68	White British	1.82	81.2	24.5	normal	never	no	no
DRH076	M	54	White British	1.72	73.7	24.9	normal	never	no	no
DRH077	M	30	White British	1.76	81.2	26.2	normal	ex	no	no
DRH078	M	56	White British	1.86	81.2	23.5	normal	never	no	no
DRH080	F	41	White British	1.68	91	32.2	normal	current	no	no
DRH081	M	32	White British	1.73	76.7	25.6	normal	never	yes	no
DRH087	M	32	White British	1.95	78.9	20.7	normal	never	no	no
DRH088	M	42	White British	1.71	93.7	32.0	normal	never	no	no
DRH089	M	65	White British	1.78	101	31.9	normal	never	no	no
DRH092	M	40	White British	1.8	117	36.1	normal	never	no	no
DRH093	M	44	White British	1.6	67.5	26.4	normal	never	no	no
DRH094	M	49	White British	1.86	90	26.0	normal	never	no	no
DRH095	M	66	White British	1.8	79.6	24.6	normal	current	no	no
DRH100	F	37	White British	1.64	60.8	22.6	normal	ex	no	no
DRH101	F	30	Eastern European	1.71	57.2	19.6	normal	current	no	no
DRH103	F	30	White British	1.66	68	24.7	normal	never	no	no
DRH107	M	54	White British	1.9	105	29.1	normal	ex	no	no
DRH108	F	29	White British	1.66	62.8	22.8	normal	never	no	no
DRH109	M	75	White British	1.7	75	26.0	normal	never	no	no
IHC048	M	74	White British	1.75	79.4	25.9	normal	never	no	no
IHC049	M	72	White British	1.75	66	21.6	normal	never	no	no

IHC054	M	28	White British	1.73	82.4	27.5	normal	never	no	no
IHC059	M	70	White British	1.8	70	21.6	normal	never	no	no
IHC061	M	47	White British	1.9	93	25.8	normal	never	no	no
IHC062	M	52	White British	1.66	79	28.7	normal	never	no	no
IHC072	M	32	White British	1.77	91	29.0	normal	current	no	no
IHC073	F	57	White British	1.62	114.4	43.6	normal	never	no	no
IHC102	M	49	White British	1.61	54	20.8	normal	never	no	no
IHC103	M	55	White British	1.86	81.3	23.5	normal	never	no	no
IHC104	M	56	White British	1.71	67	22.9	normal	current	no	no
IHC110	F	31	White British	1.57	71.4	29.0	normal	ex	no	no
IHC114	M	65	White British	1.74	78.5	25.9	normal	never	no	no
IHC115	M	31	White British	1.83	103.4	30.9	normal	ex	no	no
IHC116	M	58	White British	1.79	59.2	18.5	normal	never	no	no
IHC117	M	70	White British	1.74	99.8	33.0	normal	never	no	no
IHC120	M	59	White British	1.79	120	37.5	normal	ex	no	no
IHC121	M	57	White British	1.74	82.2	27.2	normal	never	no	no
IHC123	M	33	White British	1.79	112	35.0	normal	never	no	no
IHC124	M	60	White British	1.83	80.6	24.1	normal	never	no	no
IHC131	M	34	White British	1.77	71.4	22.8	normal	current	no	no
IHC138	F	56	White British	1.66	60	21.8	normal	never	no	no
IHC139	F	49	White British	1.69	73.2	25.6	normal	current	no	no
IHC140	F	30	White British	1.78	85.8	27.1	normal	current	no	no
IHC141	M	83	White British	1.73	71.6	23.9	normal	never	no	no
IHC142	F	42	White British	1.63	72	27.1	normal	never	no	no
IHC143	F	44	White British	1.63	89.6	33.7	normal	never	no	no
IHC144	F	39	White British	1.62	112.5	42.9	normal	never	no	no
IHC147	F	58	White British	1.67	62.2	22.3	normal	never	no	no
IHC148	M	53	White British	1.75	85.2	27.8	normal	never	no	no
IHC149	M	66	White British	1.78	99	31.2	normal	never	no	no
IHC150	M	60	White British	1.81	96.3	29.4	normal	never	no	no
IHC151	M	71	White British	1.69	86.6	30.3	normal	never	no	no

IHC152	M	62	White British	1.69	76.5	26.8	normal	ex	no	no
IHC153	M	66	White British	1.78	84.5	26.7	normal	never	no	no
IHC177	M	51	White British	1.74	78.4	25.9	normal	never	no	no
IHC178	M	72	White British	1.85	90.6	26.5	normal	never	no	no

8.2.2 IBD Patients									
Label	**Sex**	**DOB**	**Age at time of sample**	**Nationality**	**Age at diagnosis**	**Dx**	**Dx date**	**Symptoms date**	**Study Group**
DRH001A	M	19/09/1953	58	White British	54	UC	08/07/2007	01/03/2007	B,L,S
DRH001B	M	19/09/1953	58	White British	54	UC	08/07/2007	01/03/2007	B,L,S
DRH001C	M	19/09/1953	59	White British	54	UC	08/07/2007	01/03/2007	B,L,S
DRH001D	M	19/09/1953	59	White British	54	UC	08/07/2007	01/03/2007	B,L,S
DRH001E	M	19/09/1953	59	White British	54	UC	08/07/2007	01/03/2007	B,L,S
DRH001F	M	19/09/1953	59	White British	54	UC	08/07/2007	01/03/2007	B,L,S
DRH001G	M	19/09/1953	59	White British	54	UC	08/07/2007	01/03/2007	B,L,S
DRH002A	F	10/11/1966	41	White British	43	UC	16/04/2009	01/06/2009	L
DRH002B	F	10/11/1966	45	White British	43	UC	16/04/2009	01/06/2009	L
DRH002C	F	10/11/1966	46	White British	43	UC	16/04/2009	01/06/2009	L
DRH002D	F	10/11/1966	46	White British	43	UC	16/04/2009	01/06/2009	L
DRH003A	M	04/02/1981	26	White British	29	UC	30/09/2010	01/03/2010	L
DRH003B	M	04/02/1981	30	White British	29	UC	30/09/2010	01/03/2010	L
DRH003C	M	04/02/1981	31	White British	29	UC	30/09/2010	01/03/2010	L
DRH003D	M	04/02/1981	31	White British	29	UC	30/09/2010	01/03/2010	L
DRH004A	F	22/02/1982	25	Australasian	29	UC	23/08/2011	01/01/2006	L
DRH004B	F	22/02/1982	29	Australasian	29	UC	23/08/2011	01/01/2006	L
DRH004C	F	22/02/1982	30	Australasian	29	UC	23/08/2011	01/01/2006	L
DRH005A	F	11/04/1969	38	White British	34	CD	01/01/2003	unknown	B
DRH005B	F	11/04/1969	38	White British	34	CD	01/01/2003	unknown	B
DRH006A	F	04/01/1980	27	White British	23	UC	18/12/2003	01/11/2003	L
DRH006B	F	04/01/1980	31	White British	23	UC	19/12/2003	02/11/2003	L
DRH006C	F	04/01/1980	32	White British	23	UC	20/12/2003	03/11/2003	L
DRH006D	F	04/01/1980	32	White British	23	UC	21/12/2003	04/11/2003	L
DRH007A	M	09/06/1961	46	White British	38	CD	03/11/1999	01/04/1999	L
DRH007B	M	09/06/1961	50	White British	38	CD	03/11/1999	01/04/1999	L
DRH007C	M	09/06/1961	51	White British	38	CD	03/11/1999	01/04/1999	L
DRH007D	M	09/06/1961	51	White British	38	CD	03/11/1999	01/04/1999	L
DRH008A	M	09/05/1989	22	White British	22	CD	29/08/2011	01/06/2011	L

DRH008BS	M	09/05/1989	22	White British	22	CD	29/08/2011	01/06/2011	L
DRH008BU	M	09/05/1989	22	White British	22	CD	29/08/2011	01/06/2011	L
DRH008C	M	09/05/1989	23	White British	22	CD	29/08/2011	01/06/2011	L
DRH008D	M	09/05/1989	23	White British	22	CD	29/08/2011	01/06/2011	L
DRH009A	M	13/10/1988	23	White British	17	UC	29/03/2005	01/11/2004	L
DRH009B	M	13/10/1988	23	White British	17	UC	29/03/2005	01/11/2004	L
DRH009C	M	13/10/1988	24	White British	17	UC	29/03/2005	01/11/2004	L
DRH009D	M	13/10/1988	24	White British	17	UC	29/03/2005	01/11/2004	L
DRH010A	F	17/10/1949	58	White British	49	UC	07/07/1998	01/01/1990	L
DRH010B	F	17/10/1949	63	White British	49	UC	07/07/1998	01/01/1990	L
DRH010C	F	17/10/1949	63	White British	49	UC	07/07/1998	01/01/1990	L
DRH010D	F	17/10/1949	63	White British	49	UC	07/07/1998	01/01/1990	L
DRH011A	F	25/09/1991	16	White British	20	CD	25/08/2011	01/07/2011	L
DRH011B	F	25/09/1991	20	White British	20	CD	25/08/2011	01/07/2011	L
DRH011C	F	25/09/1991	21	White British	20	CD	25/08/2011	01/07/2011	L
DRH011D	F	25/09/1991	21	White British	20	CD	25/08/2011	01/07/2011	L
DRH012A	F	17/01/1965	42	White British	46	UC	02/02/2011	01/12/2010	L
DRH012B	F	17/01/1965	46	White British	46	UC	02/02/2011	01/12/2010	L
DRH012C	F	17/01/1965	47	White British	46	UC	02/02/2011	01/12/2010	L
DRH012D	F	17/01/1965	47	White British	46	UC	02/02/2011	01/12/2010	L
DRH013A	F	09/02/1952	55	White British	52	UC	06/05/2004	01/02/2004	L
DRH013B	F	09/02/1952	60	White British	52	UC	06/05/2004	01/02/2004	L
DRH013C	F	09/02/1952	60	White British	52	UC	06/05/2004	01/02/2004	L
DRH013D	F	09/02/1952	60	White British	52	UC	06/05/2004	01/02/2004	L
DRH014A	M	23/04/1960	47	White British	42	CD	01/01/2002	unknown	L,B
DRH014B	M	23/04/1960	47	White British	42	CD	01/01/2002	unknown	L,B
DRH014C	M	23/04/1960	51	White British	42	CD	01/01/2002	unknown	L,B
DRH014D	M	23/04/1960	52	White British	42	CD	01/01/2002	unknown	L,B
DRH014E	M	23/04/1960	52	White British	42	CD	01/01/2002	unknown	L,B
DRH015A	F	12/07/1945	62	White British	50	UC	28/03/1995	unknown	L
DRH015B	F	12/07/1945	66	White British	50	UC	28/03/1995	unknown	L
DRH015C	F	12/07/1945	67	White British	50	UC	28/03/1995	unknown	L
DRH015D	F	12/07/1945	67	White British	50	UC	28/03/1995	unknown	L
DRH016A	M	22/03/1939	68	White British	46	CD	01/01/1985	unknown	L

DRH016B	M	22/03/1939	73	White British	46	CD	01/01/1985	unknown	L
DRH016C	M	22/03/1939	73	White British	46	CD	01/01/1985	unknown	L
DRH016D	M	22/03/1939	73	White British	46	CD	01/01/1985	unknown	L
DRH017A	F	20/11/1967	40	White British	37	CD	01/01/2004	unknown	L,B
DRH017B	F	20/11/1967	40	White British	37	CD	01/01/2004	unknown	L,B
DRH017C	F	20/11/1967	45	White British	37	CD	01/01/2004	unknown	L,B
DRH017D	F	20/11/1967	45	White British	37	CD	01/01/2004	unknown	L,B
DRH017E	F	20/11/1967	45	White British	37	CD	01/01/2004	unknown	L,B
DRH018A	F	06/02/1976	31	White British	35	CD	30/03/2011	01/01/2008	L,B,S
DRH018B	F	06/02/1976	31	White British	35	CD	30/03/2011	01/01/2008	L,B,S
DRH018C	F	06/02/1976	36	White British	35	CD	30/03/2011	01/01/2008	L,B,S
DRH018D	F	06/02/1976	36	White British	35	CD	30/03/2011	01/01/2008	L,B,S
DRH018E	F	06/02/1976	37	White British	35	CD	30/03/2011	01/01/2008	L,B,S
DRH019A	F	10/04/1948	59	White British	62	UC	20/04/2010	unknown	S
DRH019B	F	11/04/1948	63	White British	62	UC	20/04/2010	unknown	S
DRH020A	M	15/09/1973	34	White British	31	CD	01/04/2004	01/04/2004	L
DRH020B	M	15/09/1973	39	White British	31	CD	01/04/2004	01/04/2004	L
DRH020C	M	15/09/1973	39	White British	31	CD	01/04/2004	01/04/2004	L
DRH020D	M	15/09/1973	39	White British	31	CD	01/04/2004	01/04/2004	L
DRH021A	F	15/06/1948	59	White British	63	UC	07/02/2011	unknown	L,B
DRH021B	F	15/06/1948	59	White British	63	UC	07/02/2011	unknown	L,B
DRH022A	F	01/12/1967	40	White British	40	CD	01/01/2007	unknown	L
DRH022B	F	01/12/1967	45	White British	40	CD	01/01/2007	unknown	L
DRH022C	F	01/12/1967	45	White British	40	CD	01/01/2007	unknown	L
DRH022D	F	01/12/1967	45	White British	40	CD	01/01/2007	unknown	L
DRH023A	M	23/09/1981	26	White British	25	UC	01/01/2006	unknown	S
DRH023B	M	23/09/1981	31	White British	25	UC	01/01/2006	unknown	S
DRH024A	M	14/02/1979	28	White British	25	CD	01/01/2004	unknown	L,B
DRH024B	M	14/02/1979	32	White British	25	CD	01/01/2004	unknown	L,B
DRH024C	M	14/02/1979	33	White British	25	CD	01/01/2004	unknown	L,B
DRH024D	M	14/02/1979	33	White British	25	CD	01/01/2004	unknown	L,B
DRH024E	M	14/02/1979	33	White British	25	CD	01/01/2004	unknown	L,B
DRH025A	M	31/10/1962	45	White British	46	UC	21/05/2008	unknown	L,B
DRH025B	M	31/10/1962	50	White British	46	UC	21/05/2008	unknown	L,B

DRH025C	M	31/10/1962	50	White British	46	UC	21/05/2008	unknown	L,B
DRH025D	M	31/10/1962	50	White British	46	UC	21/05/2008	unknown	L,B
DRH025E	M	31/10/1962	50	White British	46	UC	21/05/2008	unknown	L,B
DRH026A	M	25/03/1952	55	White British	55	CD	01/01/2007	unknown	L
DRH026B	M	25/03/1952	60	White British	55	CD	01/01/2007	unknown	L
DRH026C	M	25/03/1952	60	White British	55	CD	01/01/2007	unknown	L
DRH026E	M	25/03/1952	60	White British	55	CD	01/01/2007	unknown	L
DRH027A	F	07/10/1981	26	White British	26	CD	25/03/2007	unknown	L
DRH027B	F	07/10/1981	31	White British	26	CD	25/03/2007	unknown	L
DRH027C	F	07/10/1981	31	White British	26	CD	25/03/2007	unknown	L
DRH027D	F	07/10/1981	31	White British	26	CD	25/03/2007	unknown	L
DRH028A	F	07/12/1978	33	White British	26	CD	01/01/2004	unknown	S
DRH028B	F	07/12/1978	34	White British	26	CD	01/01/2004	unknown	S
DRH029A	F	13/03/1988	23	White British	22	UC	18/02/2010	unknown	L
DRH029B	F	13/03/1988	24	White British	22	UC	18/02/2010	unknown	L
DRH029C	F	13/03/1988	24	White British	22	UC	18/02/2010	unknown	L
DRH029D	F	13/03/1988	24	White British	22	UC	18/02/2010	unknown	L
DRH030A	F	17/10/1952	59	White British	57	CD	30/10/2009	unknown	L
DRH030B	F	17/10/1952	60	White British	57	CD	30/10/2009	unknown	L
DRH030C	F	17/10/1952	60	White British	57	CD	30/10/2009	unknown	L
DRH030D	F	17/10/1952	60	White British	57	CD	30/10/2009	unknown	L
DRH031A	F	03/03/1954	57	White British	57	CD	07/05/2011	unknown	L,B
DRH031B	F	03/03/1954	57	White British	57	CD	07/05/2011	unknown	L,B
DRH031C	F	03/03/1954	58	White British	57	CD	07/05/2011	unknown	L,B
DRH031D	F	03/03/1954	58	White British	57	CD	07/05/2011	unknown	L,B
DRH031E	F	03/03/1954	58	White British	57	CD	07/05/2011	unknown	L,B
DRH032A	F	04/03/1965	46	White British	42	CD	01/01/2007	unknown	L
DRH032B	F	04/03/1965	47	White British	42	CD	01/01/2007	unknown	L
DRH032C	F	04/03/1965	47	White British	42	CD	01/01/2007	unknown	L
DRH033A	F	07/03/1969	42	White British	36	UC	01/01/2005	unknown	L
DRH033B	F	07/03/1969	43	White British	36	UC	01/01/2005	unknown	L
DRH033C	F	07/03/1969	43	White British	36	UC	01/01/2005	unknown	L
DRH033D	F	07/03/1969	43	White British	36	UC	01/01/2005	unknown	L
DRH034A	M	25/07/1959	52	White British	48	UC	08/05/2007	unknown	L

DRH034B	M	25/07/1959	53	White British	48	UC	08/05/2007	unknown	L
DRH034C	M	25/07/1959	53	White British	48	UC	08/05/2007	unknown	L
DRH034D	M	25/07/1959	53	White British	48	UC	08/05/2007	unknown	L
DRH035A	F	07/04/1950	61	White British	31	CD	01/01/1981	unknown	L
DRH035B	F	07/04/1950	62	White British	31	CD	01/01/1981	unknown	L
DRH035C	F	07/04/1950	62	White British	31	CD	01/01/1981	unknown	L
DRH035D	F	07/04/1950	62	White British	31	CD	01/01/1981	unknown	L
DRH036A	F	30/09/1942	69	White British	65	UC	02/02/2007	unknown	L
DRH036B	F	30/09/1942	70	White British	65	UC	02/02/2007	unknown	L
DRH036C	F	30/09/1942	70	White British	65	UC	02/02/2007	unknown	L
DRH036D	F	30/09/1942	70	White British	65	UC	02/02/2007	unknown	L
DRH037A	F	01/03/1972	39	White British	33	UC	01/01/2005	unknown	L
DRH037B	F	01/03/1972	40	White British	33	UC	01/01/2005	unknown	L
DRH037C	F	01/03/1972	40	White British	33	UC	01/01/2005	unknown	L
DRH037D	F	01/03/1972	40	White British	33	UC	01/01/2005	unknown	L
DRH038A	F	11/12/1973	38	White British	37	UC	01/02/2010	unknown	L
DRH038B	F	11/12/1973	39	White British	37	UC	01/02/2010	unknown	L
DRH038C	F	11/12/1973	39	White British	37	UC	01/02/2010	unknown	L
DRH039A	F	13/11/1959	52	White British	24	CD	01/01/1983	unknown	L,B
DRH039B	F	13/11/1959	53	White British	24	CD	01/01/1983	unknown	L,B
DRH039C	F	13/11/1959	53	White British	24	CD	01/01/1983	unknown	L,B
DRH039D	F	13/11/1959	53	White British	24	CD	01/01/1983	unknown	L,B
DRH039E	F	13/11/1959	54	White British	24	CD	01/01/1983	unknown	L,B
DRH039F	F	13/11/1959	54	White British	24	CD	01/01/1983	unknown	L,B
DRH040A	F	02/01/1983	28	White British	7	CD	02/05/1990	unknown	S
DRH040B	F	02/01/1983	29	White British	7	CD	02/05/1990	unknown	S
DRH041A	M	19/03/1953	58	White British	52	UC	01/01/2005	unknown	L
DRH041B	M	19/03/1953	59	White British	52	UC	01/01/2005	unknown	L
DRH041C	M	19/03/1953	59	White British	52	UC	01/01/2005	unknown	L
DRH041D	M	19/03/1953	59	White British	52	UC	01/01/2005	unknown	L
DRH042A	F	26/05/1973	38	White British	35	UC	01/01/2008	unknown	L,S
DRH042B	F	26/05/1973	39	White British	35	UC	01/01/2008	unknown	L,S
DRH042C	F	26/05/1973	39	White British	35	UC	01/01/2008	unknown	L,S
DRH042D	F	26/05/1973	39	White British	35	UC	01/01/2008	unknown	L,S

DRH042E	F	26/05/1973	39	White British	35	UC	01/01/2008	unknown	L,S
DRH042F	F	26/05/1973	40	White British	35	UC	01/01/2008	unknown	L,S
DRH043A	F	22/05/1981	30	White British	29	UC	09/11/2010	unknown	L
DRH043B	F	22/05/1981	31	White British	29	UC	09/11/2010	unknown	L
DRH043C	F	22/05/1981	31	White British	29	UC	09/11/2010	unknown	L
DRH043D	F	22/05/1981	32	White British	29	UC	09/11/2010	unknown	L
DRH044A	M	01/06/1975	36	White British	32	CD	01/01/2007	unknown	L,B
DRH044B	M	01/06/1975	37	White British	32	CD	01/01/2007	unknown	L,B
DRH045A	F	23/12/1989	23	White British	20	UC	01/01/2009	unknown	S
DRH045B	F	23/12/1989	23	White British	20	UC	01/01/2009	unknown	S
DRH046A	F	15/02/1977	35	White British	35	CD	08/01/2012	unknown	Naïve to L, B
DRH046B	F	15/02/1977	35	White British	35	CD	08/01/2012	unknown	Naïve to L, B
DRH047A	F	15/05/1948	64	White British	52	UC	01/01/2000	unknown	L
DRH047B	F	15/05/1948	64	White British	52	UC	01/01/2000	unknown	L
DRH047C	F	15/05/1948	64	White British	52	UC	01/01/2000	unknown	L
DRH047D	F	15/05/1948	64	White British	52	UC	01/01/2000	unknown	L
DRH048A	F	15/02/1965	47	White British	37	CD	01/01/2002	unknown	S
DRH048B	F	15/02/1965	47	White British	37	CD	01/01/2002	unknown	S
DRH049A	F	07/03/1985	27	White British	26	CD	01/09/2011	unknown	B,S
DRH049B	F	07/03/1985	27	White British	26	CD	01/09/2011	unknown	B,S
DRH049C	F	07/03/1985	27	White British	26	CD	01/09/2011	unknown	B,S
DRH049D	F	07/03/1985	28	White British	26	CD	01/09/2011	unknown	B,S
DRH050A	M	02/10/1972	40	White British	38	CD	01/11/2010	unknown	B
DRH050B	M	02/10/1972	40	White British	38	CD	01/11/2010	unknown	B
DRH051A	M	30/10/1978	34	White British	22	CD	03/04/2000	unknown	S
DRH051B	M	30/10/1978	34	White British	22	CD	03/04/2000	unknown	S
DRH052A	F	07/01/1983	29	White British	26	UC	10/07/2009	unknown	S
DRH052B	F	07/01/1983	29	White British	26	UC	10/07/2009	unknown	S
DRH053A	F	09/06/1972	40	White British	10	CD	01/01/1982	unknown	S
DRH053B	F	09/06/1972	40	White British	10	CD	02/01/1982	unknown	S
DRH054A	F	16/07/1962	50	White British	50	CD	01/02/2012	unknown	S
DRH055A	M	12/11/1978	34	White British	33	UC	01/12/2011	unknown	S
DRH055B	M	12/11/1978	34	White British	33	UC	01/12/2011	unknown	S
DRH056A	F	26/12/1981	31	White British	28	UC	27/08/2009	unknown	S

DRH057A	F	20/06/1975	37	White British	24	CD	01/01/1999	unknown	S
DRH057B	F	20/06/1975	37	White British	24	CD	01/01/1999	unknown	S
DRH058A	M	01/05/1946	66	White British	65	CD	07/06/2011	unknown	S
DRH058B	M	01/05/1946	66	White British	65	CD	07/06/2011	unknown	S
DRH059A	F	06/01/1969	43	White British	43	UC	11/05/2012	unknown	Naïve to L
DRH059B	F	06/01/1969	43	White British	43	UC	11/05/2012	unknown	Naïve to L
DRH060A	F	21/09/1969	43	White British	22	UC	01/01/1991	unknown	B
DRH060B	F	21/09/1969	43	White British	22	UC	01/01/1991	unknown	B
DRH063A	M	09/01/1937	75	Southern European	68	UC	28/11/2005	unknown	B
DRH063B	M	09/01/1937	75	Southern European	68	UC	28/11/2005	unknown	B
DRH066A	M	05/01/1983	29	White British	29	CD	14/06/2012	unknown	S
DRH066B	M	05/01/1983	29	White British	29	CD	14/06/2012	unknown	S
DRH067A	M	09/01/1935	77	White British	77	UC	15/06/2012	unknown	S
DRH068A	F	18/02/1993	19	White British	17	UC	15/12/2010	unknown	B
DRH068B	F	18/02/1993	19	White British	17	UC	15/12/2010	unknown	B
DRH071A	F	18/03/1992	20	White British	20	CD	17/07/2012	01/01/2008	Naïve to long
DRH071B	F	18/03/1992	20	White British	20	CD	17/07/2012	01/01/2008	Naïve to long
DRH071C	F	18/03/1992	21	White British	20	CD	17/07/2012	01/01/2008	Naïve to long
DRH072A	F	15/03/1977	35	White British	28	CD	01/01/2005	unknown	S
DRH072B	F	15/03/1977	35	White British	28	CD	01/01/2005	unknown	S
DRH074A	M	25/12/1953	59	White British	59	Diverticular fistula	13/08/2012	unknown	Naïve to long
DRH075A	F	17/09/1943	69	White British	46	CD	01/01/1989	unknown	S
DRH075B	F	17/09/1943	69	White British	46	CD	01/01/1989	unknown	S
DRH079A	F	02/04/1966	46	White British	39	UC	01/01/2005	unknown	S
DRH079B	F	02/04/1966	46	White British	39	UC	01/01/2005	unknown	S
DRH082A	M	30/09/1984	28	White British	25	UC	01/01/2009	unknown	S
DRH082B	M	30/09/1984	28	White British	25	UC	01/01/2009	unknown	S
DRH083A	M	18/06/1952	60	White British	58	CD	24/11/2010	unknown	B
DRH083B	M	18/06/1952	60	White British	58	CD	24/11/2010	unknown	B
DRH084A	F	23/08/1971	41	White British	28	UC	01/01/1999	unknown	B
DRH084B	F	23/08/1971	41	White British	28	UC	01/01/1999	unknown	B
DRH085A	F	14/12/1959	53	White British	52	CD	01/01/2011	unknown	S
DRH085B	F	14/12/1959	54	White British	52	CD	01/01/2011	unknown	S
DRH086A	M	16/12/1947	65	White British	65	UC	31/10/2012	unknown	Naïve to long

DRH086B	M	16/12/1947	66	White British	65	UC	31/10/2012	unknown	Naïve to long
DRH090A	M	05/02/1973	39	White British	39	CD	06/11/2012	unknown	Naïve to long
DRH090B	M	05/02/1973	40	White British	39	CD	06/11/2012	unknown	Naïve to long
DRH091A	F	03/08/1964	48	White British	33	UC	01/01/1997	unknown	B
DRH091B	F	03/08/1964	48	White British	33	UC	01/01/1997	unknown	B
DRH096A	F	16/05/1973	39	White British	35	UC	01/01/2008	unknown	S
DRH096B	F	16/05/1973	40	White British	35	UC	01/01/2008	unknown	S
DRH097A	M	05/08/1975	37	White British	26	CD	01/08/2001	unknown	S
DRH097B	M	05/08/1975	38	White British	26	CD	01/08/2001	unknown	S
DRH098A	F	19/01/1955	57	White British		UC	unknown	unknown	B,S
DRH098B	F	19/01/1955	58	White British		UC	unknown	unknown	B,S
DRH098C	F	19/01/1955	58	White British		UC	unknown	unknown	B,S
DRH099A	F	30/01/1967	45	White British	29	CD	22/01/1996	unknown	S
DRH099B	F	30/01/1967	45	White British	29	CD	22/01/1996	unknown	S
DRH102A	M	07/06/1949	63	White British	46	CD	01/01/1995	unknown	S
DRH102B	M	07/06/1949	64	White British	46	CD	01/01/1995	unknown	S
DRH104A	F	29/11/1988	25	White British	8	CD	01/01/1996	unknown	B
DRH104B	F	29/11/1988	25	White British	8	CD	01/01/1996	unknown	B
DRH105A	F	18/08/1950	63	White British	47	UC	01/01/1997	unknown	B
DRH105B	F	18/08/1950	63	White British	47	UC	01/01/1997	unknown	B
DRH106A	M	30/07/1979	34	White British	26	CD	01/01/2005	unknown	B
DRH106B	M	30/07/1979	34	White British	26	CD	01/01/2005	unknown	B
DRH200A	M	25/10/1978	35	White British	31	CD	01/01/2009	unknown	S
DRH200B	M	25/10/1978	35	White British	31	CD	01/01/2009	unknown	S

8.2.2 IBD Patients cont…											
Label	**Sample date**	**Serum**	**Urine**	**Sample**	**Height (m)**	**Weight (kg)**	**BMI**	**Diet**	**Smoking**	**Alcohol / wk (u)**	**Drugs**
DRH001A	12/08/2011	1	1	1st long	1.69	71.2	24.9	normal	ex	0	pred 15, 6mp 50
DRH001B	15/11/2011	1	1	2nd long	1.69	72	25.2	normal	ex	0	pred 10, 6mp 50
DRH001C	08/02/2012	1	1	3rd long, pre bio	1.69	70	24.5	normal	ex	0	pred 25, 6mp 75
DRH001D	22/02/2012	1	1	post bio	1.69	73.5	25.7	normal	ex	0	pred 25, 6mp 75
DRH001E	10/05/2012	1	0	4th long	1.69	67.5	23.6	normal	ex	0	6mp 75, inflix 400
DRH001F	19/06/2012	1	1	pre surg	1.69	66.3	23.2	normal	ex	0	6mp 75, inflix 400
DRH001G	31/08/2012	1	1	post surg	1.69	71.2	24.9	normal	ex	0	tamsulosin, zopiclone, paracetamol
DRH002A	16/08/2007	1	1	Ist long	1.69	72.1	25.2	normal	never	0	6mp 75, Thyroxine 75mg OD, Agnus Castus OD (for PMS), Multivit
DRH002B	17/11/2011	1	1	2nd long	1.69	71.1	24.9	normal	never	0	6mp 100, Thyroxine 75mg OD, Agnus Castus OD (for PMS), Multivit

DRH002C	17/02/2012	1	1	3rd long	1.69	73.6	25.8	normal	never	0	citalopram, 6mp 100, Thyroxine 75mg OD, Agnus Castus OD (for PMS), Multivit
DRH002D	24/05/2012	1	1	4th long	1.69	73	25.6	normal	never	0	citalopram, 6mp 75, Thyroxine 75mg OD, Agnus Castus OD (for PMS), Multivit
DRH003A	22/08/2007	1	1	Ist long	1.7	73.4	25.4	normal	never	10	40 adalimumab every 2 weeks
DRH003B	22/11/2011	1	1	2nd long	1.7	71.4	24.7	normal	never	10	40 adalimumab every 2 weeks
DRH003C	27/02/2012	1	1	3rd long	1.7	71.6	24.8	normal	never	10	40 adalimumab every 2 weeks
DRH003D	29/05/2012	1	1	4th long	1.7	70.8	24.5	normal	never	10	40 adalimumab every 2 weeks
DRH004A	23/08/2007	1	1	Ist long	1.7	78	27.0	normal	current	0	hydrocortisone iv 400mg/day, 6mp 75mg/day, pentasa 2g po OD, Vit D /Calcium 2 tabs od, Alendronate 70mg weekly (tues), Nicotine patch in situ at present
DRH004B	25/11/2011	1	1	2nd long	1.7	84.7	29.3	normal	current	0	6mp 75
DRH004C	16/04/2012	1	1	3rd long	1.7	84	29.1	normal	current	0	6mp 75, pentasa 2gPO OD

DRH005A	23/11/2007	1	1	Pre bio	1.63	68.7	25.9	normal	current	5	Questran Light (colestyramine) 2 sachets OD
DRH005B	06/09/2007	1	1	post bio	1.63	68.7	25.9	normal	current	5	160mg adalimumab, Questran Light (colestyramine) 2 sachets OD
DRH006A	25/08/2007	1	1	Ist long	1.52	66	28.6	normal	ex	0	Movicol 1 sachet OD, methotrexate 25mg weekly, folic acid 15mg weekly, pentasa supp 1g twice / wk
DRH006B	25/11/2011	1	1	2nd long	1.52	66.3	28.7	normal	ex	0	Movicol 1 sachet OD, methotrexate 25mg weekly, folic acid 15mg weekly, pentasa supp 1g twice / wk
DRH006C	02/03/2012	1	1	3rd long	1.52	67	29.0	normal	ex	0	Movicol 1 sachet OD, methotrexate 25mg weekly, folic acid 15mg weekly, pentasa supp 1g twice / wk, diazepam

DRH006D	01/06/2012	1	1	4th long	1.52	65.6	28.4	normal	ex	0	Movicol 1 sachet OD, methotrexate 25mg weekly, folic acid 15mg weekly, pentasa supp 1g twice / wk, antibiotics for ear infection (?what)
DRH007A	29/08/2007	1	1	Ist long	1.81	65.3	20.0	normal	current	0	pred 35mg od, calcichew
DRH007B	25/11/2011	1	1	2nd long	1.81	74	22.6	normal	current	0	pred 30mg od, calcichew
DRH007C	24/02/2012	1	1	3rd long	1.81	72.5	22.16	normal	current	0	pred 15mg od, calcichew
DRH007D	25/05/2012	1	1	4th long	1.81	72.6	22.2	normal	current	0	pred 30mg od, calcichew
DRH008A	30/08/3007	1	1	Ist long	1.85	75.4	22.0	normal	ex	0	hydrocortisone iv 400mg/day, ranitidine 150mg po bd, enoxaparin 20mg sc od, pabrinex I+II bd iv, zopiclone 7.5mg od po, peptac 10ml po nocte, dihydrocodeine 60mg nocte
DRH008BS	23/11/2011	1	0	2nd long serum only	1.85	83.7	24.5	normal	ex	0	infliximab 400mg/8 weeks

DRH008BU	14/12/2011	0	1	2nd long urine only	1.85	83.7	24.5	normal	ex	0	infliximab 400mg/8 weeks
DRH008C	28/03/2012	1	1	3rd long	1.85	83	24.3	normal	current	0	infliximab 400mg/8 weeks
DRH008D	20/06/2012	1	1	4th long	1.85	82.7	24.1636	normal	current	0	infliximab 400mg/8 weeks
DRH009A	01/09/2007	1	1	Ist long	1.74	80.4	26.6	normal	never	0	6mp 50
DRH009B	08/12/2011	1	1	2nd long	1.74	84.5	28.0	normal	never	0	6mp 50
DRH009C	08/03/2012	1	1	3rd long	1.74	83.8	27.7	normal	never	0	6mp 50
DRH009D	07/06/2012	1	1	4th long	1.74	83.7	27.6	normal	never	0	6mp 50
DRH010A	05/09/2007	1	1	Ist long	1.68	70.6	25.0	normal	never	0	40mg adalimumab 2 weekly
DRH010B	10/01/2012	1	1	2nd long	1.68	72	25.5	normal	never	0	40mg adalimumab 2 weekly
DRH010C	27/03/2012	1	1	3rd long	1.68	72.5	25.7	normal	never	0	40mg adalimumab 2 weekly
DRH010D	22/06/2012	1	1	4th long	1.68	74	26.2	normal	never	0	40mg adalimumab 2 weekly
DRH011A	07/09/2007	1	1	Ist long	1.66	78	28.3	normal	never	0	400mg infliximab, 6mp 50, alcium, vitamin d, microgynon 30
DRH011B	25/11/2011	1	1	2nd long	1.66	80.9	29.4	normal	never	0	400mg infliximab, 6mp 50, alcium, vitamin d, microgynon 30

DRH011C	15/02/2012	1	1	3rd long	1.66	86	31.2	normal	never	0	400mg infliximab, 6mp 50, alcium, vitamin d, microgynon 31
DRH011D	06/06/2012	1	1	4th long	1.66	86	31.2	normal	never	0	400mg infliximab, 6mp 50, alcium, vitamin d, microgynon 32
DRH012A	07/09/2007	1	1	Ist long	1.54	56	23.6	normal	ex	14	pred 30, 6mp 50
DRH012B	14/12/2011	1	1	2nd long	1.54	55.3	23.3	normal	ex	14	infliximab 300mg / 8 weeks
DRH012C	22/03/2012	1	1	3rd long	1.54	54	22.8	normal	ex	14	nil
DRH012D	14/08/2012	1	1	4th long	1.54	53.8	22.7	normal	ex	14	nicotine gum
DRH013A	08/09/2007	1	1	Ist long	1.58	64.4	25.8	normal	ex	0	pentasa 1g po BD, pentasa 1g supp every 2nd day, lansoprazole 30mg od, folic acid, levothyroxine 75mcg od, cyanocobalamin 50mcg od, zopiclone 7.5mg prn

DRH013B	18/01/2012	1	1	2nd long	1.58	66.4	26.6	normal	ex	0	pentasa 1g po BD, pentasa 1g supp every 2nd day, lansoprazole 30mg od, folic acid, levothyroxine 75mcg od, cyanocobalamin 50mcg od, zopiclone 7.5mg prn
DRH013C	06/07/2012	1	1	3rd long	1.58	65	26.0	normal	ex	0	pentasa 1g po BD, pentasa 1g supp every 2nd day, lansoprazole 30mg od, folic acid, levothyroxine 75mcg od, cyanocobalamin 50mcg od, zopiclone 7.5mg prn
DRH013D	15/10/2012	1	1	4th long	1.58	64.6	25.9	normal	ex	0	pentasa 1g po BD, pentasa 1g supp every 2nd day, lansoprazole 30mg od, folic acid, levothyroxine 75mcg od, cyanocobalamin 50mcg od, zopiclone 7.5mg prn
DRH014A	19/09/2007	1	1	1st long	1.76	111.9	36.1	normal	ex	30	azathioprine 200mg od, amlodipine 5mg od, enoxaparin 20mg

DRH014B	05/10/2007	1	1	pre bio	1.76	111.9	36.1	normal	ex	30	azathioprine 200mg od, amlodipine 5mg od
DRH014C	12/12/2011	1	1	2nd long	1.76	113.4	36.6	normal	ex	30	azathioprine 200mg od, amlodipine 5mg od, inliximab 500mg 8 weekly
DRH014D	14/03/2012	1	1	3rd long	1.76	110	35.5	normal	ex	30	azathioprine 200mg od, amlodipine 5mg od, infliximab 500mg 8 weekly
DRH014E	05/07/2012	1	1	4th long	1.76	111.2	35.9	normal	ex	30	azathioprine 200mg od, amlodipine 5mg od
DRH015A	26/09/2007	1	1	1st long	1.65	70.6	25.9	normal	ex	0	asacol 800mg po bd, aloe vera tablets, loperamide prn, omeprazole, salbutamol 100mcg inh prn, budesonide 200mcg 2 puffs bd,
DRH015B	14/12/2011	1	1	2nd long	1.65	70.3	25.8	normal	ex	0	asacol 800mg po bd, aloe vera tablets, loperamide prn, omeprazole, salbutamol 100mcg inh prn, budesonide 200mcg 2 puffs bd,

DRH015C	14/03/2012	1	1	3rd long	1.65	68.6	25.2	normal	ex	0	asacol 800mg po bd, aloe vera tablets, loperamide prn, omeprazole, salbutamol 100mcg inh prn, budesonide 200mcg 2 puffs bd,
DRH015D	21/06/2012	1	1	4th long	1.65	70.5	25.9	normal	ex	0	asacol 800mg po bd, aloe vera tablets, loperamide prn, omeprazole, salbutamol 100mcg inh prn, budesonide 200mcg 2 puffs bd,
DRH016A	02/10/2007	1	1	1st long	1.64	81	30.1	normal	ex	1	mercaptopurine 75mg od, lansoprazole 30mg od
DRH016B	09/01/2012	1	1	2nd long	1.64	82.2	30.6	normal	ex	1	mercaptopurine 50mg od, lansoprazole 30mg od
DRH016C	02/04/2012	1	1	3rd long	1.64	81.8	30.4	normal	ex	1	mercaptopurine 100mg od, lansoprazole 30mg od
DRH016D	09/07/2012	1	1	4th long	1.64	81.3	30.2	normal	ex	1	mercaptopurine 100mg od, lansoprazole 30mg od
DRH017A	03/10/2007	1	1	1st long, pre bio	1.63	85	32.0	normal	never	0	nil

DRH017B	17/10/2007	1	1	post bio	1.63	84	31.6	normal	never	0	400mg infliximab 2 weeks ago
DRH017C	11/01/2012	1	1	2nd long	1.63	87	32.7	normal	never	0	400mg infliximab 8 weekly, 200mg azathioprine
DRH017D	03/04/2012	1	1	3rd long	1.63	89	33.5	normal	never	0	400mg infliximab 8 weekly, 200mg azathioprine
DRH017E	22/08/2012	1	1	4th long	1.63	85	32.0	normal	never	0	400mg infliximab 8 weekly, 200mg azathioprine
DRH018A	03/10/2007	1	1	1st long, pre bio	1.57	48	19.5	normal	never	0	oramorph, morphine iv, cyclizine, dihydrocodeine, procalopride 1mg od, paracetamol iv,
DRH018B	19/10/2007	1	1	post bio	1.57	48.5	19.7	normal	never	0	oramorph, cyclizine, dihydrocodeine, procalopride 1mg od, paracetamol, infliximab 250mg 2 weeks ago
DRH018C	01/02/2012	1	1	2nd long	1.57	50	20.3	normal	never	0	oramorph, cyclizine, dihydrocodeine, procalopride 1mg od, paracetamol, infliximab 250mg 8 weekly

DRH018D	06/09/2012	1	1	pre surgery, 3rd long	1.57	52.4	21.3	TPN	never	0	oramorph, cyclizine, dihydrocodeine, procalopride 1mg od, paracetamol, infliximab 250mg 8 weekly
DRH018E	08/02/2013	1	1	post surgery, 4th long	1.57	52	21.1	normal	never	0	oramorph, cyclizine, dihydrocodeine, procalopride 1mg od, paracetamol, infliximab 250mg 8 weekly
DRH019A	11/10/2007	1	1	pre surgery	1.57	64	26.0	normal	current	10	loperamide prn
DRH019B	02/12/2011	1	1	post surgery	1.57	58.6	23.8	normal	current	10	preload at 1800/2200 11/10/11, exoxapain at 17:00 11/10/11
DRH020A	12/10/2007	1	1	1st long	1.79	69	21.5	normal	never	5	Nil
DRH020B	10/01/2012	1	1	2nd long	1.79	69	21.5	normal	never	5	Nil
DRH020C	30/03/2012	1	1	3rd long	1.79	66.2	20.7	normal	never	5	Nil
DRH020D	06/07/2012	1	1	4th long	1.79	67.5	21.1	normal	never	5	Nil
DRH021A	17/10/2007	1	1	1st long, pre bio	1.59	59	23.3	normal	never	0	prednisolone 5mg, mercaptopurine 50mg od, amitryptyline 25mg bd, picolax x 2 17/10/11

DRH021B	01/11/2007	1	1	post bio	1.59	59	23.3	normal	never	0	mercaptopurine 50mg od, amitryptyline 25mg bd, infliximab 300mg 2 weeks ago
DRH022A	18/10/2007	1	1	1st long	1.65	50.5	18.5	normal	current	5	picolax x2, folic acid 15mg od, omeprazole 20mg od, ascorbic acid, co-codamol prn
DRH022B	23/01/2012	1	1	2nd long	1.65	50.2	18.4	normal	current	5	folic acid 15mg od, omeprazole 20mg od, ascorbic acid, co-codamol prn
DRH022C	31/05/2012	1	1	3rd long	1.65	50.8	18.7	normal	current	5	folic acid 15mg od, omeprazole 20mg od, ascorbic acid, co-codamol prn
DRH022D	02/10/2012	1	1	4th long	1.65	49.3	18.1	normal	current	5	folic acid 15mg od, omeprazole 20mg od, ascorbic acid, co-codamol prn

DRH023A	25/10/2007	1	1	pre surg	1.78	75.2	23.7	normal	never	0	prednisolone 9mg. Azathioprine 125mg od, pentasa 2g bd, calcium and vit d supplements 1 tab bd, cocodamol prn, enoxaparin 40mg pre-op and preload 10pm and 6am
DRH023B	10/03/2012	1	1	post surg	1.78	71	22.4	normal	never	0	
DRH024A	01/11/2007	1	1	1st long, pre bio	1.69	70	24.5	normal	never	0	folic acid 5mg / week, ibuprofen 400mg prn.
DRH024B	15/11/2011	1	1	post bio	1.69	70	24.5	normal	never	0	folic acid 5mg / week, ibuprofen 400mg prn, infliximab 400mg 2 weeks ago
DRH024C	08/02/2012	1	1	2nd long	1.69	70	24.5	normal	never	0	folic acid 5mg / week, ibuprofen 400mg prn, infliximab 400mg 8 weekly
DRH024D	30/05/2012	1	1	3rd long	1.69	66	23.1	normal	never	0	folic acid 5mg / week, ibuprofen 400mg prn, infliximab 400mg 8 weekly
DRH024E	19/09/2012	1	1	4th long	1.69	68	23.8	normal	never	0	folic acid 5mg / week, ibuprofen 400mg prn, infliximab 400mg 8

											weekly
DRH025A	03/11/2007	1	1	1st long	1.75	76.5	25.0	normal	ex	40	prednisolone 15mg od, mercaptopurine 50mg od
DRH025B	27/01/2012	1	1	2nd long	1.75	80.4	26.3	normal	ex	40	mercaptopurine 75mg od
DRH025C	22/02/2012	1	1	pre bio	1.75	78	25.5	normal	ex	40	mercaptopurine 75mg od
DRH025D	07/03/2012	1	1	post bio, 3rd long	1.75	76	24.8	normal	current	40	infliximab 400mg 2 weeks ago
DRH025E	30/05/2012	1	1	4th long	1.75	76	24.8	normal	current	40	infliximab 400mg 8 weekly
DRH026A	03/11/2007	1	1	1st long	1.75	102	33.3	normal	ex	70	adalimumab 40mg every 2 weeks, tramadol 50mg prn, diclofenac 50mg prn, tadalifil prn, zydol xl prn,
DRH026B	27/01/2012	1	1	2nd long	1.75	102.5	33.5	normal	ex	70	adalimumab 80mg every 2 weeks
DRH026C	02/05/2012	1	1	3rd long	1.75	101.5	33.1	normal	ex	70	adalimumab 80mg every 2 weeks

DRH026E	15/08/2012	1	1	4th long	1.75	103	33.6	normal	ex	70	tacrolimus 2mg bd, buprenorphine 10mcg patch, prednisolone 40mg 13/08/12 only, tramadol 50mg prn, omeprazole, diclofenac
DRH027A	06/11/2007	1	1	1st long	1.59	64.6	25.6	normal	current	5	adalimumab 40mg every 2 weeks
DRH027B	30/01/2012	1	1	2nd long	1.59	63.2	25.0	normal	current	5	adalimumab 40mg every 2 weeks
DRH027C	09/05/2012	1	1	3rd long	1.59	63.6	25.2	normal	current	5	adalimumab 40mg every 2 weeks
DRH027D	21/09/2012	1	1	4th long	1.59	61.8	24.4	normal	ex	0	adalimumab 40mg every 2 weeks
DRH028A	07/11/2011	1	1	pre surg	1.65	55.4	20.3	normal	ex	0	co-codamol prn, tramadol prn, enoxaparin 20mg, preload, venofer 200mg iv 4/10/11
DRH028B	02/03/2012	1	1	post surg	1.65	56.2	20.6	normal	ex	0	co-codamol prn, tramadol prn.
DRH029A	08/11/2011	1	1	1st long	1.77	68.9	22.0	normal	never	10	adalimumab 40mg 2 weekly
DRH029B	01/03/2012	1	1	2nd long	1.77	67	21.4	normal	never	10	adalimumab 40mg 2 weekly
DRH029C	29/06/2012	1	1	3rd long	1.77	67.4	21.5	normal	never	10	adalimumab 40mg 2 weekly
DRH029D	28/12/2012	1	1	4th long	1.77	68.8	22.0	normal	never	10	adalimumab 40mg weekly

DRH030A	08/11/2011	1	1	1st long	1.64	79.6	29.6	normal	never	0	mercaptopurine 75mg od, lansoprazole
DRH030B	08/02/2012	1	1	2nd long	1.64	78	29.0	normal	never	0	mercaptopurine 75mg od, lansoprazole
DRH030C	08/05/2012	1	1	3rd long	1.64	76.8	28.6	normal	never	0	mercaptopurine 75mg od, lansoprazole
DRH030D	20/08/2012	1	1	4th long	1.64	77.8	28.9	normal	never	0	mercaptopurine 75mg od, lansoprazole
DRH031A	08/11/2011	1	1	1st long, pre bio	1.64	86.9	32.3	normal	ex	0	levothyroxine 50mcg od, paroxetine 30mg od, bendroflumethiazide 2.5mg od, ramipril 10mg od, atenolol 50mg of, metformin 500mg bd, simvastatin 40mg od, novorapid as per BM, lantus as per BM.

DRH031B	23/11/2011	1	1	post bio	1.64	92.7	34.5	normal	ex	0	levothyroxine 50mcg od, paroxetine 30mg od, bendroflumethiazide 2.5mg od, ramipril 10mg od, atenolol 50mg of, metformin 500mg bd, simvastatin 40mg od, novorapid as per BM, lantus as per BM, infliximab 400mg 2 weeks ago.
DRH031C	20/02/2012	1	1	2nd long	1.64	86	32.0	normal	ex	0	levothyroxine 50mcg od, paroxetine 30mg od, bendroflumethiazide 2.5mg od, ramipril 10mg od, atenolol 50mg of, metformin 500mg bd, simvastatin 40mg od, novorapid as per BM, lantus as per BM, infliximab 400mg 8 weekly.

DRH031D	05/06/2012	1	1	3rd long	1.64	94.4	35.1	normal	ex	0	levothyroxine 50mcg od, paroxetine 30mg od, bendroflumethiazide 2.5mg od, ramipril 10mg od, atenolol 50mg of, metformin 500mg bd, simvastatin 40mg od, novorapid as per BM, lantus as per BM, infliximab 400mg 8 weekly.
DRH031E	02/10/2012	1	1	4th long	1.64	94	34.9	normal	ex	0	levothyroxine 50mcg od, paroxetine 30mg od, bendroflumethiazide 2.5mg od, ramipril 10mg od, atenolol 50mg of, metformin 500mg bd, simvastatin 40mg od, novorapid as per BM, lantus as per BM, picolax, infliximab
DRH032A	15/11/2011	1	1	1st long	1.56	88	36.2	normal	ex	0	azathioprine150mg od, bisoprolol 5m od

DRH032B	14/02/2012	1	1	2nd long	1.56	88	36.2	normal	ex	2	azathioprine150mg od, bisoprolol 5m od
DRH032C	17/05/2012	1	1	3rd long	1.56	89	36.6	normal	ex	2	azathioprine150mg od, bisoprolol 5m od
DRH033A	15/11/2011	1	1	1st long	1.64	63.6	23.6	normal	ex	2	pentasa 1g bd, carbomazepine 100mg mane, 200mg nocte, omeprazole 20mg od, movicol prn
DRH033B	21/02/2012	1	1	2nd long	1.64	63.4	23.6	normal	ex	2	pentasa 1g bd, carbomazepine 100mg mane, 200mg nocte, omeprazole 20mg od, movicol prn

DRH033C	05/06/2012	1	1	3rd long	1.64	61.3	22.8	normal	ex	2	pentasa 1g bd, carbomazepine 100mg mane, 200mg nocte, omeprazole 20mg od, movicol prn, picolax
DRH033D	28/08/2012	1	1	4th long	1.64	62.1	23.1	normal	ex	2	pentasa 1g bd, carbomazepine 100mg mane, 200mg nocte, omeprazole 20mg od, movicol prn
DRH034A	17/11/2011	1	1	1st long	1.84	93.4	27.6	normal	never	10	pentasa enemas prn
DRH034B	13/02/2012	1	1	2nd long	1.84	92.9	27.4	normal	never	10	pentasa enemas prn
DRH034C	14/05/2012	1	1	3rd long	1.84	92.8	27.4	normal	never	10	pentasa enemas prn
DRH034D	31/08/2012	1	1	4th long	1.84	95	28.1	normal	never	10	pentasa enemas prn
DRH035A	21/11/2011	1	1	1st long	1.56	56	23.0	vegetarian	never	0	azathioprine100mg od, multivitamin tablet od
DRH035B	16/02/2012	1	1	2nd long	1.56	56	23.0	vegetarian	never	0	azathioprine100mg od, multivitamin tablet od
DRH035C	18/05/2012	1	1	3rd long	1.56	54.6	22.4	vegetarian	never	0	azathioprine100mg od, multivitamin tablet od
DRH035D	06/09/2012	1	1	4th long	1.56	54.8	22.5	vegetarian	never	0	azathioprine100mg od, multivitamin tablet od

DRH036A	22/11/2011	1	1	1st long	1.58	62.5	25.0	normal	ex	5	mercaptopurine 50mg od, thyroxine 75mcg od.
DRH036B	28/02/2012	1	1	2nd long	1.58	62	24.8	normal	ex	5	mercaptopurine 50mg od, thyroxine 75mcg od.
DRH036C	28/05/2012	1	1	3rd long	1.58	61.3	24.6	normal	ex	5	mercaptopurine 50mg od, thyroxine 75mcg od.
DRH036D	05/09/2012	1	1	4th long	1.58	60.8	24.6	normal	ex	5	mercaptopurine 50mg od, thyroxine 75mcg od.
DRH037A	23/11/2011	1	1	1st long	1.72	87	29.4	normal	current	10	pentasa 2g od
DRH037B	14/02/2012	1	1	2nd long	1.72	88.6	30.0	normal	current	5	pentasa 2g od
DRH037C	15/05/2012	1	1	3rd long	1.72	89	30.1	normal	current	5	pentasa 2g od
DRH037D	14/08/2012	1	1	4th long	1.72	86.2	29.1	normal	current	5	pentasa 2g od
DRH038A	23/11/2011	1	1	1st long	1.59	84	33.2	vegetarian	ex	0	pentasa supps prn
DRH038B	20/02/2012	1	1	2nd long	1.59	80.7	31.9	vegetarian	ex	0	pentasa supps prn
DRH038C	25/05/2012	1	1	3rd long	1.59	81.4	32.2	vegetarian	ex	0	pentasa supps prn
DRH039A	28/11/2011	1	1	1st long	1.6	58.4	22.8	normal	never	4	mercaptopurine 50mg od
DRH039B	28/02/2012	1	1	2nd long	1.6	60.2	23.5	normal	never	4	mercaptopurine 50mg od
DRH039C	28/06/2012	1	1	3rd long	1.6	60	23.4	normal	never	4	mercaptopurine 50mg od

DRH039D	09/10/2012	1	1	4th long	1.6	60	23.4	normal	never	4	Nil
DRH039E	17/01/2013	1	1	pre bio	1.6	60	23.4	normal	never	4	Nil
DRH039F	31/01/2013	1	1	post bio	1.6	59	23.0	normal	never	4	adalimumab 160mg 2 weeks ago
DRH040A	30/11/2011	1	1	pre surg	1.61	50	19.3	normal	never	0	adalimumab 40mg every 2 weeks
DRH040B	02/03/2012	1	1	post surg	1.61	50.2	19.4	normal	never	0	adalimumab 40mg every 2 weeks
DRH041A	07/12/2011	1	1	1st long	1.74	77	25.4	normal	ex	12	prednisolone 35mg od, asacol 2.4g od
DRH041B	13/03/2012	1	1	2nd long	1.74	76.2	25.2	normal	ex	12	methotrexate 25mg weekly
DRH041C	06/07/2012	1	1	3rd long	1.74	77.4	25.6	normal	ex	12	methotrexate 25mg weekly
DRH041D	25/10/2012	1	1	4th long	1.74	76.6	25.3	normal	ex	12	methotrexate 25mg weekly
DRH042A	12/12/2011	1	1	1st long	1.57	69.7	28.3	normal	ex	1	pentasa 2g bd, pentasa supps prn, mebeverine 135mg prn
DRH042B	08/03/2012	1	1	2nd long	1.57	70.6	28.6	normal	ex	1	pentasa 2g bd, pentasa supps prn, mebeverine 135mg prn
DRH042C	08/06/2012	1	1	3rd long	1.57	71.6	29.0	normal	ex	1	prednisolone 10mg od, mercaptopurine 50mg od

DRH042D	21/09/2012	1	1	4th long	1.57	69.4	28.2	normal	ex	1	mercaptopurine 75mg od, infliximab 400mg every 8 weeks
DRH042E	12/12/2012	1	1	pre surg	1.57	70	28.4	normal	ex	0	mercaptopurine 75mg od, infliximab 400mg every 8 weeks
DRH042F	25/02/2013	1	1	post surg	1.57	70	28.4	normal	ex	0	
DRH043A	14/12/2011	1	1	1st long	1.73	73	24.4	normal	never	14	cocyprindiol(ocp), pentasa 1g supps as req,
DRH043B	08/05/2012	1	1	2nd long	1.73	72.6	24.3	normal	never	14	cocyprindiol(ocp), pentasa 1g supps as req,
DRH043C	23/10/2012	1	1	3rd long	1.73	70.6	23.6	normal	never	14	cocyprindiol(ocp), pentasa 1g supps as req,
DRH043D	29/01/2013	1	1	4th long	1.73	73.5	24.6	vegetarian	never	14	cocyprindiol(ocp), pentasa 1g supps as req, fluconase nasal spray + antihistamine
DRH044A	15/12/2011	1	1	1st long, pre bio	1.85	59.6	17.4	normal	current	0	warfarin, tramadol, codeine, lansoprazole, cyclizine,

DRH044B	04/01/2012	1	0	post bio	1.85	56	16.4	normal	current	0	warfarin, tramadol, codeine, lansoprazole, cyclizine, inflizimab 300mg given 2 weeks ago
DRH045A	05/01/2012	1	1	pre surg	1.63	65	24.5	normal	never	0	mercaptopurine 75mg / 50mg alternating days, adcal d3 tab bd, ranitidine 300mg nocte, prednisolone 25mg od, ondansetron 8mg nocte, enoxaparin 20mg sc, paracetamol 1g, on iv 0.9%NaCl
DRH045B	02/03/2012	1	1	post surg	1.63	59.8	22.5	normal	never	0	ranitidine 150mg, paracetamol 1g
DRH046A	08/01/2012	1	1	1st naïve to long, pre bio	1.49	54.2	24.4	normal	current	0	
DRH046B	02/02/2012	1	1	post bio	1.49	53.4	24.1	normal	current	0	prednisolone 20mg od, mercaptopurine 75mg od, infliximab 400mg 2 weeks ago
DRH047A	10/01/2012	1	1	1st long	1.64	68.5	25.5	normal	never	0	asacol 800mg bd, bendroflumethiazide, lisinopril, simvastatin

DRH047B	08/05/2012	1	1	2nd long	1.64	67	24.9	normal	never	0	asacol 800mg bd, bendroflumethiazide, lisinopril, simvastatin
DRH047C	20/08/2012	1	1	3rd long	1.64	65.4	24.3	normal	never	0	asacol 800mg bd, bendroflumethiazide, lisinopril, simvastatin
DRH047D	29/11/2012	1	1	4th long	1.64	65	24.2	normal	never	0	asacol 800mg bd, bendroflumethiazide, lisinopril, simvastatin
DRH048A	17/01/2012	1	1	pre surg	1.65	64	23.5	normal	current	0	azathioprine 150mg od, picolax, oxynorm, oxycodone, dihydrocodeine, omeprazole, hydroxycobalamin 1mg 3 monthly, adcal d3 2 tabs od, diazepam 5mg tds, salbutamol inh, seretide inh, citalopram 40mg od, paracetamol 1g prn, zolpdem 10mg nocte, livial 2mg od.

DRH048B	05/04/2012	1	1	post surg	1.65	64	23.5	normal	current	0	azathioprine 150mg od, picolax, oxynorm, oxycodone, dihydrocodeine, omeprazole, hydroxycobalamin 1mg 3 monthly, adcal d3 2 tabs od, diazepam 5mg tds, salbutamol inh, seretide inh, citalopram 40mg od, paracetamol 1g prn, zolpdem 10mg nocte, livial 2mg od.
DRH049A	17/01/2012	1	1	pre bio	1.74	67.4	22.3	no red meat	ex	2	budesonide 9mg od, salbutamol inh, budesonide inh
DRH049B	31/01/2012	1	1	post bio	1.74	67.7	22.4	no red meat	ex	2	budesonide 9mg od, salbutamol inh, budesonide inh, adalimumab 160mg 2 weeks ago
DRH049C	07/12/2012	1	1	pre surg	1.74	52.5	17.3	no red meat	ex	0	infliximab 400mg 8 weekly, diazepam 5mg, pabrinex I+II, enoxaparin 40mg, cerazette (ocp), omeprazole 20mg

DRH049D	29/01/2013	1	1	post surg	1.74	46.8	15.5	normal	ex	0	oxycodone, hyosciene, omeprazole, cyclizine
DRH050A	18/01/2012	1	1	pre bio	1.75	88.2	28.8	normal	ex	0	methotrexate 25 mg / week, folic acid 15mg/wk
DRH050B	01/02/2012	1	1	post bio	1.75	88	28.7	normal	ex	0	infliximab 400mg 2 weeks ago, folic acid 15mg/week
DRH051A	19/01/2012	1	1	pre surg	1.78	78.3	24.7	normal	never	6	mercaptopurine 50mg od, adalimumab 40mg 2 weekly, questran 1 sachet od
DRH051B	23/03/2012	1	1	post surg	1.78	76.2	24.1	normal	never	6	mercaptopurine 50mg od, adalimumab 40mg 2 weekly, questran 1 sachet od
DRH052A	07/02/2012	1	1	pre surg	1.61	62	23.9	normal	never	0	enoxaparin 40mg, sando k 2 tabs

DRH052B	02/05/2012	1	1	post surg	1.61	52.2	20.1	normal	never	0	loratidine, cholphenamine, amytriptyline, singular, tramadol, enoxaparin
DRH053A	29/02/2012	1	1	pre surg	1.59	83.6	33.1	normal	never	4	paracetamol 1g qds, gentamicin 24/02/12, metronidazole 21/2/12 - 2/3/12, sando k 23-26/2/12, tazocin 24/2/12-2/3/12, enoxaparin 40mg, oramorph 10mg prn, ondansetron 4mg prn, cyclizine 50mg prn
DRH053B	04/04/2012	1	1	post surg	1.59	82	32.4	normal	never	4	adalimumab 160mg 2 weeks ago

DRH054A	29/01/2012	1	1	pre surg	1.62	87	33.2	normal	current	0	picolax x 2 20/02/2012, infliximab 500mg 22/2/12, hydrocortisone 200mg iv 20/2/12, enoxaparin 40mg, paracetamol 1g qds, omeprazole 40mg od, cefuroxime 1.5g iv 15+16/2/12, metronidazole 500mg iv 15+16/2/12, tramadol 50mg prn, cyclizine 50mg prn, buscopan 20mg 20/2/12
DRH055A	20/03/2012	1	1	pre surg	1.88	66	18.7	normal	ex	0	mercaptopurine 75mg od, infliximab 400mg 8 weekly
DRH055B	11/05/2012	1	1	post surg	1.88	71.4	20.2	normal	ex	0	Nil
DRH056A	28/03/2012	1	1	pre surg	1.64	67.8	25.2	normal	never	1	yasmin, citalopram 20mg, dosulepin 25mg
DRH057A	30/03/2012	1	1	pre surg	1.57	47	19.1	normal	ex	0	prednisolone 2mg

DRH057B	25/05/2012	1	1	post surg	1.57	47.2	19.1	gluten free	ex	0	dapsone 50mg od, antihistamine
DRH058A	29/03/2012	1	1	pre surg	1.8	68.3	21.2	normal	current	0	gliclazide, simvastatin, pioglitazone, phor sandoz, aspirin, mirtazipine, metformin, inflix 2011 - 09/02/2012 (300mg)
DRH058B	15/06/2012	1	1	post surg	1.8	71.3	22.0	normal	current	0	patient states nil?
DRH059A	11/05/2012	1	1	1st naïve to long	1.61	61.9	23.9	normal	current	10	Nil
DRH059B	26/10/2012	1	0	2nd long	1.61	66.2	25.5	normal	current	10	Nil
DRH060A	23/05/2012	1	1	pre bio	1.68	97	34.4	normal	ex	0	prednisolone 25mg od, olsalazine 1g bd, omeprazole, predsol supps
DRH060B	07/06/2012	1	1	post bio	1.68	105	37.2	normal	ex	0	infliximab 400mg 2 weeks ago, olsalazine 1g bd, omeprazole, predsol supps
DRH063A	06/06/2012	1	1	pre bio	1.62	98	37.3	normal	ex	0	pentasa 1g bd

DRH063B	20/06/2012	1	1	post bio	1.62	98	37.3	normal	ex	0	infliximab 400mg 2 weeks ago, pentasa 1g bd
DRH066A	27/06/2012	1	1	pre surg	1.91	79	21.7	normal	current	20	Nil
DRH066B	17/09/2012	1	1	post surg	1.91	83	22.8	normal	current	20	omeprazole, paracetamol
DRH067A	28/06/2012	1	1	pre surg	1.76	71	22.9	normal	ex	7	hydrocortisone 400mg iv, tazocin, enoxaparin, omeprazole, ferrous sulphate
DRH068A	05/07/2012	1	1	pre bio	1.64	80	29.7	normal	never	0	prednisolone 40mg od, mercaptopurine 75mg od
DRH068B	18/07/2012	1	1	post bio	1.64	85	31.6	normal	never	0	prednisolone 10mg, mercaptopurine 75mg od, infliximab 400mg 2 weeks ago
DRH071A	17/07/2012	1	1	1st Naïve to long	1.66	59.8	21.7	normal	never	0	picolax x 2, diclofenac, paracetamol
DRH071B	01/10/2012	1	1	2nd long	1.66	60.3	21.9	normal	never	0	mercaptopurine 50mg od

DRH071C	18/01/2013	1	1	3rd long	1.66	59.4	21.6	normal	never	0	mercaptopurine 50mg od
DRH072A	19/07/2012	1	1	pre surg	1.61	63.4	24.5	normal	ex	0	enoxaparin 40mg
DRH072B	01/11/2012	1	1	post surg	1.61	61.7	23.8	normal	ex	0	Nil
DRH074A	14/08/2012	1	1	1st naïve to long	1.77	105	33.5	normal	current	0	omeprazole 10mg, tazocin
DRH075A	15/08/2012	1	1	pre surg	1.7	67	23.2	normal	current	0	furosemide 20mg temazepam 20mg clonidine 75mg MST 10mg bd aspirin ec 75mg) oramorph nifedipine la 20mg quinine sulphate 300mg peppermint oil mebeverine 135mg pizotifen 1.5mg diazepam 2mg folic acid 5mg GTN spray prn sulfasalazine 1.5g bd simvastatin 20mg amitriptyline 75mg

DRH075B	27/11/2012	1	1	post surg	1.7	67.1	23.2	normal	current	0	furosemide 20mg temazepam 20mg clonidine 75mg MST 10mg bd clopidogrel 75mg oramorph nifedipine la 20mg quinine sulphate 300mg peppermint oil mebeverine 135mg pizotifen 1.5mg diazepam 2mg folic acid 5mg GTN spray prn sulfasalazine 1g bd simvastatin 40mg amitriptyline 50mg
DRH079A	02/07/2012	1	1	pre surg	1.65	76	27.9	normal	ex	15	metformin, lisinopril, salbutamol, gliclazide, ferrous sulphate, forceval
DRH079B	14/09/2012	1	1	post surg	1.65	81.3	29.9	normal	current	15	metformin, lisinopril, salbutamol, gliclazide, ferrous sulphate, forceval
DRH082A	21/08/2012	1	1	pre surg	1.81	85	25.9	normal	never	0	infliximab 400mg 8 weekly, tacroliomus 5mg mane, 6mg nocte pentasa 2g bd enoxaparin
DRH082B	29/10/2012	1	1	post surg	1.81	86.6	26.4	normal	never	10	Nil

DRH083A	28/08/2012	1	1	pre bio	1.79	79.3	24.7	normal	never	0	prednisolone 2mg, asacol 1.2g bd
DRH083B	12/09/2012	1	1	post bio	1.79	78	24.3	normal	never	0	prednisolone 2mg, asacol 1.2g bd, adalimumab 160mg 2 weeks ago
DRH084A	20/09/2012	1	1	pre bio	1.65	78	28.7	normal	ex	0	prednisolone 30mg valganciclovir 900mg bd azathioprine 175mg od ompreazole 20mg od adcal d3 2 tabs anusol prn paracetamol / dihydrocodeine prn
DRH084B	04/10/2012	1	1	post bio	1.65	78	28.7	normal	ex	0	prednisolone 20mg valganciclovir 900mg od azathioprine 175mg od ompreazole 20mg od adcal d3 2 tabs anusol prn paracetamol / dihydrocodeine prn, infliximab 400mg 2 weeks ago
DRH085A	21/09/2012	1	1	pre surg	1.52	61.6	26.7	normal	current	10	azathioprine 125mg od
DRH085B	17/01/2013	1	1	post surg	1.52	67.8	29.3	normal	current	10	azathioprine 125mg od

DRH086A	31/10/2012	1	1	1st naïve to long	1.68	92.6	32.8	normal	never	5	lisinopril, amlodipine, simvastatin, PO4 enema, midazolam, vit K 10mg iv
DRH086B	12/02/2013	1	1	2nd long	1.68	90	31.9	normal	never	5	lisinopril, amlodipine, simvastatin, prednisolone 5mg
DRH090A	06/11/2012	1	1	1st naïve to long	1.78	68	21.5	low residue diet	never	2	Paracetamol
DRH090B	19/01/2013	1	1	2nd long	1.78	74	23.4	low residue diet	never	2	prednisolone 10mg od, mercaptopurine 100mg od
DRH091A	14/11/2012	1	1	pre bio	1.63	76.2	28.7	no red meat	never	0	Nil
DRH091B	28/11/2012	1	1	post bio	1.63	72	27.1	no red meat	never	0	infliximab 400mg 2 weeks ago
DRH096A	30/11/2012	1	1	pre surg	1.7	102.9	35.6	vegetarian	ex	0	azathioprine 200mg od, olsalazine 1g, adcal d3, amitriptyline 25, omprazole 20mg, cilest 1
DRH096B	01/02/2013	1	1	post surg	1.7	91.2	31.6	vegetarian	ex	0	omeprazole, amitriptyline, cilest

DRH097A	11/12/2012	1	1	pre surg	1.73	87	29.1	normal	ex	0	mercaptopurine 100mg od, adalimumab 40mg weekly, enoxaparin 40mg 10/12/12, preload 18:00 + 22:00 10/12/12
DRH097B	01/02/2013	1	1	post surg	1.73	81.7	27.3	normal	ex	0	vitamin tablets
DRH098A	12/12/2012	1	1	pre bio	1.66	95	34.5	normal	never	0	aspirin 75mg od, mesalazine ec 200mg qds, rosouvastatin 10mg od, omeprazole 20mg od, furosemide 20mg od, hyoscine butylbromide 10mg prn, codeine 30mg prn, xyloprost 5% topical prep bd, paracetamol 1g qds, tramadol 100mg prn, iv hydrocortisone 400mg / 24 hours, enoxaparin 40mg

DRH098B	04/01/2013	1	1	post bio, pre surg	1.66	92	33.4	normal	never	0	preload 18:00 + 22:00, enoxaparin 40mg 18:00, aspirin 75mg, rosuvastatin 10mg, omeprazole 20mg, furosemide 20mg, xyloprost bd, co-codamol 30/500 prn, infliximab 12/12/12
DRH098C	25/02/2013	1	1	post surg	1.66	90	32.7	normal	never	0	aspirin 75mg, rosuvastatin 10mg, omeprazole 20mg, furosemide 20mg, xyloprost bd, co-codamol 30/500 prn
DRH099A	20/04/2012	1	1	pre surg	1.59	79	31.2	normal	never	0	Nil
DRH099B	04/09/2012	1	1	post surg	1.59	68.9	27.3	normal	never	0	warfarin, infliximab 400mg every 8 weeks

DRH102A	13/12/2012	1	1	pre surg	1.62	60.2	22.9	normal	ex	0	omeprazole 40mg, enoxaparin 40mg, preload 18:00, 22:00, 10mg pred od, 50 mg aza bd, folic acid 5mg od, adcal d3 1.5g tds, pentasa 500mg tds, ascorbic acid 100mg od, codeine phos 15mg qds, ranitidine 150mg od, paracetamol 1g 22:50
DRH102B	01/02/2013	1	1	post surg	1.62	61.7	23.5	normal	ex	0	adcal d3 1.5tabs, paracetamol, codeine phosphate prn, loperamide prn, vitamin tab
DRH104A	16/01/2013	1	1	pre bio	1.6	99	38.7	normal	never	5	mercaptopurine 100mg od
DRH104B	29/01/2013	1	1	post bio	1.6	97	37.9	normal	never	5	mercaptopurine 100mg od, infliximab 500mg 2 weeks ago
DRH105A	16/01/2013	1	1	pre bio	1.73	76	25.4	normal	ex	0	prednisolone 10mg od
DRH105B	30/01/2013	1	1	post bio	1.73	76	25.4	normal	ex	0	infliximab 400mg 2 weeks ago
DRH106A	17/01/2013	1	1	pre bio	1.78	60	18.9	normal	never	10	azathioprine 150mg od

DRH106B	31/01/2013	1	1	post bio	1.78	62	19.6	low residue diet	never	10	azathioprine 150mg od, infliximab 400mg 2 weeks ago
DRH200A	23/01/2013	1	1	pre surg	1.77	76.2	24.3	normal	ex	4	mercaptopurine 75mg od, paracetamol 1g qds, dihydrocodeine 30mg qds, oramorph 10mg at 23:00 22/1/13
DRH200B	25/02/2013	1	1	post surg	1.77	76	24.3	normal	ex	4	paracetamol, tramadol

8.2.2 cont…												
Label	**BO day**	**BO night**	**urgency**	**blood in stool**	**gen well being**	**arthralgia**	**pyoderma gangrenosum**	**erythythema nodosum**	**uveitis**	**paris**	**sccai**
DRH001A	1	0	nil	trace	v well	n	n	n	n	e2+	1
DRH001B	2	0	hurry	nil	poor	n	n	n	n	e2+	3
DRH001C	2	1	hurry	usually frank	poor	n	n	n	n	e2+	7
DRH001D	2	0	hurry	occasionally frank	slightly below par	n	n	n	n	e2+	4
DRH001E	3	0	hurry	occasionally frank	poor	n	n	n	n	e2+	5
DRH001F	3	0	hurry	usually frank	poor	n	n	n	n	e2+	6
DRH001G	stoma	stoma	stoma	nil	slightly below par	n	n	n	n	n/a	n/a (1)
DRH002A	2	0	nil	nil	poor	n	n	n	n	e4	2
DRH002B	1	0	nil	nil	slightly below par	n	n	n	n	e4	1
DRH002C	1	0	nil	nil	poor	n	n	n	n	e4	2
DRH002D	3	0	nil	nil	slightly below par	y	n	n	n	e4	2
DRH003A	2	0	hurry	nil	slightly below par	n	n	n	n	e4	2
DRH003B	2	0	nil	nil	slightly below par	n	n	n	n	e4	1
DRH003C	1	0	nil	nil	v well	n	n	n	n	e4	0
DRH003D	1	0	nil	nil	v well	n	n	n	n	e4	0
DRH004A	3	0	nil	nil	slightly below par	n	n	n	n	e2+	1
DRH004B	8	0	hurry	occasionally frank	slightly below par	n	n	n	n	e2+	6
DRH004C	1	0	nil	nil	v well	n	n	n	n	e2+	0
DRH005A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH005B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH006A	6	1	hurry	occasionally frank	poor	y	n	n	n	e2	8
DRH006B	2	0	nil	n	poor	y	n	n	n	e2	3
DRH006C	3	0	hurry	n	poor	y	n	n	n	e2	4
DRH006D	6	1	hurry	n	poor	y	n	n	n	e4	6
DRH007A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH007B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH007C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH007D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH008A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH008BS	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH008BU	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

DRH008C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH008D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH009A	1	0	nil	nil	slightly below par	n	n	n	n	e2	1
DRH009B	1	0	nil	nil	v well	n	n	n	n	e2	0
DRH009C	1	0	nil	nil	slightly below par	n	n	n	n	e2	1
DRH009D	1	0	nil	nil	v well	n	n	n	n	e2	0
DRH010A	3	0	nil	nil	poor	n	n	n	n	e2	2
DRH010B	2	1	hurry	occasionally frank	poor	n	n	n	n	e2	6
DRH010C	1	0	nil	occasionally frank	slightly below par	n	n	n	n	e2	3
DRH010D	3	0	hurry	occasionally frank	slightly below par	n	n	n	n	e2	4
DRH011A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH011B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH011C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH011D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH012A	15	0	incontinent	occasionally frank	poor	n	n	n	n	e2	10
DRH012B	10	0	incontinent	usually frank	terrible	n	n	n	n	e2	13
DRH012C	2	0	hurry	usually frank	v poor	n	n	n	n	e2	7
DRH012D	1	0	nil	nil	slightly below par	n	n	n	n	e2	1
DRH013A	1	0	nil	nil	very well	n	n	n	n	e1	0
DRH013B	1	0	nil	trace	slightly below par	n	n	n	n	e1	2
DRH013C	1	0	nil	trace	slightly below par	n	n	n	n	e1	2
DRH013D	1	0	nil	trace	very well	n	n	n	n	e1	1
DRH014A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH014B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH014C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH014D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH014E	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH015A	4	1	immediately	nil	poor	n	n	n	n	e2	6
DRH015B	3	0	hurry	nil	slightly below par	n	n	n	n	e2	2
DRH015C	4	0	hurry	nil	poor	n	n	n	n	e2	4
DRH015D	4	0	hurry	nil	poor	n	n	n	n	e2	4
DRH016A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH016B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH016C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

DRH016D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH017A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH017B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH017C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH017D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH017E	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH018A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH018B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH018C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH018D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH018E	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH019A	6	1	incontinent	nil	poor	n	n	n	n	e4	7
DRH019B	stoma	stoma	stoma	nil	slightly below par	n	n	n	n	n/a	1
DRH020A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH020B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH020C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH020D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH021A	2	3	hurry	usually frank	poor	n	n	n	n	e2	7
DRH021B	1	0	nil	occasionally frank	poor	n	n	n	n	e2	4
DRH022A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH022B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH022C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH022D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH023A	4	2	hurry	nil	slightly below par	n	n	n	n	e2+	4
DRH023B											
DRH024A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH024B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH024C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH024D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH024E	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH025A	2	0	hurry	nil	slightly below par	n	n	n	n	e2+	2
DRH025B	3	0	hurry	occasionally frank	slightly below par	n	n	n	n	e4	4
DRH025C	2	0	hurry	occasionally frank	slightly below par	n	n	n	n	e4	4
DRH025D	4	0	hurry	usually frank	poor	n	n	n	n	e4	7

DRH025E	6	1	immediately	usually frank	very poor	n	n	n	n	e4	10
DRH026A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH026B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH026C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH026E	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH027A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH027B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH027C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH027D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH028A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH028B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH029A	1	0	nil	nil	very well	n	n	n	n	e4	0
DRH029B	1	0	nil	nil	slightly below par	n	n	n	n	e4	1
DRH029C	6	0	hurry	usually frank	poor	n	n	n	n	e4	7
DRH029D	1	0	nil	nil	very well	n	n	n	n	e4	0
DRH030A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH030B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH030C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH030D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH031A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH031B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH031C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH031D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH031E	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH032A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH032B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH032C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH033A	1	0	nil	nil	very well	n	n	n	n	e2	0
DRH033B	1	0	nil	trace	slightly below par	n	n	n	n	e2	2
DRH033C	1	0	nil	nil	poor	n	n	n	n	e2	2
DRH033D	0	0	nil	nil	slightly below par	n	n	n	n	e2	1
DRH034A	1	0	nil	nil	very well	n	n	n	n	e2	0
DRH034B	1	0	nil	nil	slightly below par	n	n	n	n	e2	1
DRH034C	1	0	nil	nil	very well	n	n	n	n	e2	0

DRH034D	1	0	nil	nil	very well	n	n	n	n	e2	0
DRH035A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH035B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH035C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH035D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH036A	1	0	nil	nil	very well	y	n	n	n	e1	1
DRH036B	1	0	nil	nil	very well	n	n	n	n	e1	0
DRH036C	1	0	nil	nil	very well	n	n	n	n	e1	0
DRH036D	1	0	nil	nil	very well	n	n	n	n	e1	0
DRH037A	2	0	nil	nil	very well	n	n	n	n	e4	0
DRH037B	1	0	nil	nil	slightly below par	n	n	n	n	e4	1
DRH037C	1	0	nil	nil	very well	n	n	n	n	e4	0
DRH037D	1	0	nil	nil	very well	n	n	n	n	e4	0
DRH038A	2	0	nil	nil	slightly below par	n	n	n	n	e1	1
DRH038B	1	0	nil	nil	very well	n	n	n	n	e1	0
DRH038C	2	0	nil	nil	very well	n	n	n	n	e1	0
DRH039A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH039B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH039C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH039D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH039E	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH039F	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH040A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH040B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH041A	1	0	nil	nil	slightly below par	n	n	n	n	e2	1
DRH041B	1	0	nil	nil	very well	n	n	n	n	e2	0
DRH041C	1	0	nil	nil	very well	n	n	n	n	e2	0
DRH041D	1	0	nil	nil	very well	n	n	n	n	e2	0
DRH042A	2	0	nil	trace	slightly below par	n	n	n	n	e2	2
DRH042B	7	4	immediately	occasionally frank	poor	y	n	n	n	e2	11
DRH042C	2	0	nil	nil	slightly below par	n	n	n	n	e2	1
DRH042D	8	1	immediately	occasionally frank	poor	y	n	n	n	e2	10
DRH042E	10	5	immediately	trace	poor	n	n	n	n	e2	10
DRH042F	stoma	stoma	stoma	nil	very well	n	n	n	n	n/a	0(stoma)

DRH043A	1	0	nil	nil	slightly below par	n	n	n	n	e1	1
DRH043B	1	0	nil	nil	very well	n	n	n	n	e1	0
DRH043C	1	0	hurry	trace	slightly below par	n	n	n	n	e1	3
DRH043D	1	4	nil	usually frank	slightly below par	n	n	n	n	e1	4
DRH044A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH044B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH045A	20	2	immediately	trace	terrible	n	n	n	n	e4	11
DRH045B	stoma	stoma	stoma	nil	slightly below par	n	n	n	n	n/a	1(stoma)
DRH046A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH046B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH047A	1	0	nil	nil	slightly below par	n	n	n	n	e4	1
DRH047B	1	0	nil	nil	very well	n	n	n	n	e4	0
DRH047C	2	0	nil	nil	very well	n	n	n	n	e4	0
DRH047D	1	0	nil	nil	slightly below par	n	n	n	n	e4	1
DRH048A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH048B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH049A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH049B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH049C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH049D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH050A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH050B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH051A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH051B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH052A	stoma	stoma	stoma	nil	very well	n	n	n	n	n/a	0(stoma)
DRH052B	stoma	stoma	stoma	nil	slightly below par	n	n	n	n	n/a	1(stoma)
DRH053A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH053B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH054A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH055A	20	1	immediately	occasionally frank	terrible	n	n	n	n	e4	12
DRH055B	stoma	stoma	stoma	nil	slightly below par	n	n	n	n	n/a	1(stoma)
DRH056A	stoma	stoma	stoma	nil	very well	n	n	n	n	n/a	0(stoma)
DRH057A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH057B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

DRH058A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH058B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH059A	3	2	immediately	usually frank	terrible	n	n	n	n	e2+	10
DRH059B	6	0	hurry	nil	slightly below par	n	n	n	n	e2+	3
DRH060A	8	0	hurry	occasionally frank	poor	n	n	n	n	e1	7
DRH060B	4	0	nil	occasionally frank	slightly below par	n	n	n	n	e1	4
DRH063A	5	0	immediately	occasionally frank	poor	n	n	n	n	e4	7
DRH063B	3	0	hurry	nil	slightly below par	n	n	n	n	e4	2
DRH066A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH066B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH067A	20	2	immediately	usually frank	very poor	n	n	n	n	e4	12
DRH068A	20	5	hurry	occasionally frank	very poor	n	n	n	n	e4	11
DRH068B	6	0	hurry	nil	slightly below par	n	n	n	n	e4	3
DRH071A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH071B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH071C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH072A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH072B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH074A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH075A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH075B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH079A	stoma	stoma	stoma	nil	poor	n	n	n	n	n/a	2(stoma)
DRH079B	stoma	stoma	stoma	nil	very well	n	n	n	n	n/a	0(stoma)
DRH082A	20	2	immediately	usually frank	very poor	n	n	n	n	e2	12
DRH082B	stoma	stoma	stoma	nil	very well	n	n	n	n	n/a	0(stoma)
DRH083A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH083B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH084A	3	0	hurry	trace	poor	n	n	n	n	e3	4
DRH084B	2	0	hurry	nil	slightly below par	n	n	n	n	e3	2
DRH085A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH085B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH086A	10	5	immediately	usually frank	terrible	n	n	n	n	e2+	14
DRH086B	10	2	immediately	occasionally frank	very poor	n	n	n	n	e2+	11
DRH090A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

DRH090B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH091A	8	2	hurry	usually frank	very poor	y	n	n	n	e3	11
DRH091B	3	0	hurry	trace	slightly below par	y	n	n	n	e3	4
DRH096A	10	10	immediately	usually frank	terrible	y	n	n	n	e2	15
DRH096B	stoma	stoma	stoma	nil	poor	n	n	n	n	n/a	2(stoma)
DRH097A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH097B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH098A	15	5	incontinent	usually frank	very poor	n	n	n	n	e2+	14
DRH098B	10	5	hurry	occasionally frank	very poor	y	n	n	n	e2+	12
DRH098C	stoma	stoma	stoma	nil	slightly below par	n	n	n	n	n/a	1(stoma)
DRH099A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH099B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH102A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH102B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH104A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH104B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH105A	6	0	hurry	trace	slightly below par	n	n	n	n	e2	4
DRH105B	2	0	hurry	nil	slightly below par	n	n	n	n	e2	2
DRH106A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH106B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH200A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH200B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

8.2.2 cont…												
Label	**gen well**	**abdo pain**	**Liquid stool / day**	**abdo mass**	**arthralgia**	**uveitis**	**erythema nodosum**	**pyoderma gangrenosum**	**anal fissue**	**new fistula**	**abscess**	**HBI**
DRH001A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH001B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH001C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH001D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH001E	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH001F	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH001G	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH002A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH002B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH002C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH002D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH003A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH003B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH003C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH003D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH004A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH004B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH004C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH005A	slightly below par	moderate	15	nil	n	n	n	n	n	n	n	18
DRH005B	poor	mild	10	nil	n	n	n	n	n	n	n	13
DRH006A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH006B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH006C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH006D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH007A	poor	moderate	1	nil	n	n	n	n	n	n	y	6
DRH007B	slightly below par	mild	3	nil	y	n	n	n	n	n	n	6

DRH007C	terrible	severe	0	nil	n	n	n	n	n	n	n	7
DRH007D	poor	moderate	1	nil	n	n	n	n	n	n	y	6
DRH008A	very poor	mild	5	nil	n	n	n	n	n	n	n	9
DRH008BS	v well	none	0	nil	n	n	n	n	n	n	n	0
DRH008BU	v well	none	0	nil	n	n	n	n	n	n	n	0
DRH008C	slightly below par	none	2	nil	n	n	n	n	n	n	n	3
DRH008D	v well	none	1	nil	n	n	n	n	n	n	n	1
DRH009A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH009B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH009C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH009D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH010A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH010B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH010C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH010D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH011A	slightly below par	mild	2	nil	n	n	n	n	n	n	n	4
DRH011B	slightly below par	mild	8	nil	n	n	n	n	n	n	n	10
DRH011C	poor	mild	0	nil	n	n	n	n	n	n	n	3
DRH011D	poor	none	1	nil	n	n	n	n	n	n	n	3
DRH012A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH012B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH012C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH012D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH013A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH013B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH013C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH013D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH014A	slightly below	mild	7	nil	n	n	n	n	n	y	y	11

	par											
DRH014B	slightly below par	none	4	nil	n	n	n	n	n	n	n	5
DRH014C	very poor	mild	8	nil	n	n	n	n	n	n	n	12
DRH014D	poor	none	5	nil	n	n	n	n	n	n	n	7
DRH014E	very poor	moderate	6	nil	n	n	n	n	n	n	n	11
DRH015A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH015B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH015C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH015D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH016A	very well	none	2	nil	y	n	n	n	n	n	n	3
DRH016B	slightly below par	none	3	nil	n	n	n	n	n	n	n	4
DRH016C	poor	none	1	nil	n	n	n	n	n	n	n	3
DRH016D	very well	none	0	nil	n	n	n	n	n	n	n	0
DRH017A	slightly below par	moderate	1	nil	n	n	n	n	n	n	n	4
DRH017B	very well	none	0	nil	n	n	n	n	n	n	n	0
DRH017C	very well	none	0	nil	n	n	n	n	n	n	n	0
DRH017D	very well	none	0	nil	n	n	n	n	n	n	n	0
DRH017E	very well	none	0	nil	n	n	n	n	n	n	n	0
DRH018A	very poor	severe	1	nil	n	n	n	n	n	n	n	7
DRH018B	poor	mild	0	nil	n	n	n	n	n	n	n	3
DRH018C	terrible	mild	1	nil	n	n	n	n	n	n	n	6
DRH018D	poor	moderate	0	nil	n	n	n	n	n	n	n	4
DRH018E	terrible	severe	0	nil	n	n	n	n	n	n	n	7
DRH019A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH019B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH020A	slightly below par	none	stoma	nil	n	n	n	n	n	n	n	1 (stoma)
DRH020B	slightly below par	none	stoma	nil	n	n	n	n	n	n	n	1(stoma)

DRH020C	very well	none	stoma	nil	n	n	n	n	n	n	n	0(stoma)
DRH020D	very well	none	stoma	nil	n	n	n	n	n	n	n	0(stoma)
DRH021A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH021B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH022A	slightly below par	mild	4	nil	y	n	n	n	n	n	n	7
DRH022B	poor	mild	6	nil	y	n	n	n	n	n	n	10
DRH022C	slightly below par	mild	2	nil	n	n	n	n	n	n	n	4
DRH022D	poor	moderate	0	nil	n	n	n	n	n	n	n	4
DRH023A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH023B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH024A	poor	moderate	4	nil	y	n	n	n	n	n	n	9
DRH024B	slightly below par	none	4	nil	n	n	n	n	n	n	n	5
DRH024C	slightly below par	none	2	nil	n	n	n	n	n	n	n	5
DRH024D	very well	none	2	nil	n	n	n	n	n	n	n	5
DRH024E	very well	none	1	nil	n	n	n	n	n	n	n	5
DRH025A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH025B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH025C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH025D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH025E	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH026A	very well	mild	7	nil	n	n	n	n	n	n	n	8
DRH026B	slightly below par	mild	4	nil	y	n	n	n	n	n	n	7
DRH026C	slightly below par	moderate	0	nil	n	n	n	n	n	n	n	3
DRH026E	poor	moderate	6	nil	y	n	n	n	n	n	n	11
DRH027A	poor	mild	2	nil	n	n	n	n	n	n	n	5

DRH027B	slightly below par	mild	2	nil	n	n	n	n	n	n	n	4
DRH027C	slightly below par	moderate	0	nil	n	n	n	n	n	n	n	3
DRH027D	very poor	moderate	10	nil	y	n	n	n	n	n	n	16
DRH028A	very poor	moderate	stoma	nil	n	n	n	n	n	n	n	5(stoma)
DRH028B	slightly below par	none	stoma	nil	n	n	n	n	n	n	n	1(stoma)
DRH029A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH029B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH029C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH029D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH030A	very well	mild	5	nil	y	n	n	n	n	n	n	7
DRH030B	slightly below par	none	6	nil	n	n	n	n	n	n	n	7
DRH030C	very well	none	0	nil	n	n	n	n	n	n	n	0
DRH030D	very well	none	2	nil	n	n	n	n	n	n	n	2
DRH031A	slightly below par	mild	6	nil	n	n	n	n	n	n	n	8
DRH031B	slightly below par	mild	2	nil	n	n	n	n	n	n	n	4
DRH031C	poor	mild	12	nil	n	n	n	n	n	n	n	15
DRH031D	poor	moderate	4	nil	n	n	n	n	n	n	n	8
DRH031E	slightly below par	mild	4	nil	n	n	n	n	n	n	n	6
DRH032A	slightly below par	none	0	nil	n	n	n	n	n	n	n	1
DRH032B	poor	none	0	nil	n	n	n	n	n	n	n	2
DRH032C	very well	none	0	nil	n	n	n	n	n	n	n	0
DRH033A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH033B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

DRH033C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH033D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH034A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH034B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH034C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH034D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH035A	slightly below par	mild	2	nil	y	n	n	n	n	n	n	5
DRH035B	slightly below par	mild	3	nil	n	n	n	n	n	n	n	5
DRH035C	very well	none	2	nil	n	n	n	n	n	n	n	2
DRH035D	very well	none	1	nil	n	n	n	n	n	n	n	1
DRH036A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH036B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH036C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH036D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH037A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH037B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH037C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH037D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH038A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH038B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH038C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH039A	slightly below par	none	2	nil	y	n	n	n	n	n	n	4
DRH039B	slightly below par	mild	2	nil	n	n	n	n	n	n	n	4
DRH039C	poor	mild	4	nil	n	n	n	n	n	n	n	7
DRH039D	poor	mild	4	nil	y	n	n	n	n	n	n	8
DRH039E	poor	moderate	2	nil	n	n	n	n	n	n	n	6
DRH039F	slightly below par	mild	2	nil	n	n	n	n	n	n	n	4
DRH040A	very poor	moderat	stoma	nil	y	n	n	n	n	n	n	6(stoma)

		e										
DRH040B	slightly below par	mild	stoma	nil	n	n	n	n	n	n	n	2(stoma)
DRH041A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH041B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH041C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH041D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH042A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH042B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH042C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH042D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH042E	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH042F	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH043A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH043B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH043C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH043D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH044A	terrible	severe	2	dubious	y	n	n	n	n	n	n	11
DRH044B	terrible	moderate	0	nil	n	n	n	n	n	n	n	6
DRH045A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH045B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH046A	terrible	severe	20	nil	n	n	n	n	n	n	n	27
DRH046B	very poor	mild	4	nil	n	n	n	n	n	n	n	8
DRH047A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH047B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH047C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH047D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH048A	terrible	severe	3	nil	n	n	n	n	n	n	n	10
DRH048B	terrible	moderate	0	nil	n	n	n	n	n	n	n	6
DRH049A	terrible	moderate	6	nil	n	n	n	n	n	n	n	12
DRH049B	very poor	moderat	0	nil	n	n	n	n	n	n	n	5

		e										
DRH049C	terrible	mild	2	dubious	n	n	n	n	n	n	n	8
DRH049D	terrible	severe	2	nil	n	n	n	n	n	n	n	9
DRH050A	poor	none	2	nil	n	n	n	n	n	n	n	4
DRH050B	slightly below par	mild	2	nil	n	n	n	n	n	n	n	4
DRH051A	slightly below par	moderat e	0	nil	n	n	n	n	n	n	n	3
DRH051B	slightly below par	none	0	nil	n	n	n	n	n	n	n	1
DRH052A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH052B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH053A	terrible	none	0	nil	n	n	n	n	n	y	y	6
DRH053B	very well	none	stoma	nil	n	n	n	n	n	n	n	0(stoma)
DRH054A	terrible	severe	0	nil	n	n	n	n	n	n	n	7
DRH055A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH055B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH056A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH057A	terrible	severe	0	nil	n	n	n	n	n	n	n	7
DRH057B	slightly below par	none	10	nil	n	n	n	n	n	n	n	11
DRH058A	very poor	moderat e	10	nil	n	y	n	n	n	n	n	16
DRH058B	poor	none	stoma	nil	n	n	n	n	n	n	n	2(stoma)
DRH059A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH059B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH060A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH060B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH063A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH063B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH066A	very poor	severe	stoma	dubious	n	n	n	n	n	n	n	7(stoma)
DRH066B	very well	none	stoma	nil	n	n	n	n	n	n	n	0(stoma)
DRH067A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH068A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

DRH068B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH071A	poor	moderate	6	nil	y	n	y	n	n	n	n	12
DRH071B	very poor	moderate	4	nil	n	n	n	n	n	n	n	9
DRH071C	very poor	severe	8	nil	n	n	n	y	n	n	n	15
DRH072A	very poor	moderate	7	tender and definate mass	n	n	n	n	n	n	n	15
DRH072B	poor	none	10	nil	y	n	n	n	n	n	n	13
DRH074A	very poor	severe	5	dubious	y	n	n	n	n	n	n	13
DRH075A	terrible	severe	0	nil	n	n	n	n	n	n	n	7
DRH075B	poor	moderate	10	nil	n	n	n	n	n	n	n	14
DRH079A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH079B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH082A												
DRH082B												
DRH083A	poor	moderate	2	nil	y	n	n	n	n	n	n	7
DRH083B	slightly below par	mild	2	nil	n	n	n	n	n	n	n	4
DRH084A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH084B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH085A	poor	moderate	2	nil	y	n	n	n	n	y	n	8
DRH085B	very well	none	1	nil	n	n	n	n	n	n	n	1
DRH086A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH086B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH090A	very poor	moderate	4	dubious	n	n	n	n	n	n	n	10
DRH090B	slightly below par	mild	3	nil	n	n	n	n	n	n	n	5
DRH091A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH091B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

DRH096A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH096B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH097A	poor	moderate	5	nil	y	n	n	n	n	n	n	10
DRH097B	very well	none	stoma	nil	n	n	n	n	n	n	n	0(stoma)
DRH098A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH098B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH098C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH099A	very poor	moderate	10	nil	n	n	n	n	n	n	n	15
DRH099B	slightly below par	none	stoma	nil	n	n	n	n	n	n	n	1(stoma)
DRH102A	poor	moderate	2	nil	n	n	n	n	n	n	n	6
DRH102B	poor	moderate	0	nil	n	n	n	n	n	n	n	4
DRH104A	poor	moderate	4	nil	n	n	n	n	n	n	n	8
DRH104B	very well	none	0	nil	n	n	n	n	n	n	n	0
DRH105A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH105B	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
DRH106A	poor	moderate	3	nil	n	n	n	n	n	n	n	7
DRH106B	poor	mild	2	nil	n	n	n	n	n	n	n	5
DRH200A	very poor	severe	8	dubious	n	n	n	n	n	n	n	15
DRH200B	poor	moderate	6	nil	n	n	n	n	n	n	n	10

8.2.2 cont…								
Label	**location**	**behaviour**	**age**	**medical history**	**surgical history**	**date of IBD surgery (in study)**	**IBD surgery (in study)**	**reason for surgery**
DRH001A	n/a	n/a	n/a		Perianal haematoma 1990, rubber band ligation of haemorrhoids 1990, mastectomy for gynaecomastia prior to 1996	19/06/2012	colectomy	chronic colitis
DRH001B	n/a	n/a	n/a					
DRH001C	n/a	n/a	n/a					
DRH001D	n/a	n/a	n/a					
DRH001E	n/a	n/a	n/a					
DRH001F	n/a	n/a	n/a					
DRH001G	n/a	n/a	n/a					
DRH002A	n/a	n/a	n/a	hypothyroid, fatigue	tonsillectomy, c-section x 2, sterilistaion			
DRH002B	n/a	n/a	n/a	hypothyroid, fatigue				
DRH002C	n/a	n/a	n/a	hypothyroid, fatigue				
DRH002D	n/a	n/a	n/a	hypothyroid, fatigue				
DRH003A	n/a	n/a	n/a		inguinal hernia aged 5			
DRH003	n/a	n/a	n/a					

B								
DRH003C	n/a	n/a	n/a					
DRH003D	n/a	n/a	n/a					
DRH004A	n/a	n/a	n/a		3 x gynae laparoscopy for endometriosis			
DRH004B	n/a	n/a	n/a					
DRH004C	n/a	n/a	n/a					
DRH005A	L3	B1	A2		right hemicolectomy for crohns 2006			
DRH005B	L3	B1	A2					
DRH006A	n/a	n/a	n/a					
DRH006B	n/a	n/a	n/a					
DRH006C	n/a	n/a	n/a					
DRH006D	n/a	n/a	n/a					
DRH007A	L3	B2p	A2		right hemicolectomy 2003 for iatrogenic perf during stricture dilatation, open chole 2003, i&d perianal abscess 2005, eua & fistulotomy 2007, EUA and i&d ischiorectal abscess 27/3/12			
DRH007B	L3	B2p	A2					
DRH007	L3	B2p	A2					

C								
DRH007D	L3	B2p	A2					
DRH008A	L2	B1	A2			08/02/2012	i&d perianal abscess	abscess
DRH008BS	L2	B1	A2					
DRH008BU	L2	B1	A2					
DRH008C	L2	B1p	A2					
DRH008D	L2	B1p	A2					
DRH009A	n/a	n/a	n/a					
DRH009B	n/a	n/a	n/a					
DRH009C	n/a	n/a	n/a					
DRH009D	n/a	n/a	n/a					
DRH010A	n/a	n/a	n/a		lap chole 2010 cholelithiasis			
DRH010B	n/a	n/a	n/a					
DRH010C	n/a	n/a	n/a					
DRH010D	n/a	n/a	n/a					
DRH011A	L2	B1	A2					
DRH011B	L2	B1	A2					
DRH011	L2	B1	A2					

C								
DRH011 D	L2	B1	A2					
DRH012 A	n/a	n/a	n/a					
DRH012 B	n/a	n/a	n/a					
DRH012 C	n/a	n/a	n/a					
DRH012 D	n/a	n/a	n/a					
DRH013 A	n/a	n/a	n/a	hypothyroid, vit B def				
DRH013 B	n/a	n/a	n/a	hypothyroid, vit B def				
DRH013 C	n/a	n/a	n/a	hypothyroid, vit B def				
DRH013 D	n/a	n/a	n/a	hypothyroid, vit B def				
DRH014 A	L2	B1p	A3		20/9/2011 EUA, excision of fistula tract, insertion of seton suture			
DRH014 B	L2	B1p	A3		20/9/2011 EUA, excision of fistula tract, insertion of seton suture			
DRH014 C	L2	B1p	A3		20/9/2011 EUA, excision of fistula tract, insertion of seton suture			
DRH014 D	L2	B1p	A3		20/9/2011 EUA, excision of fistula tract, insertion of seton suture			
DRH014 E	L2	B1p	A3		20/9/2011 EUA, excision of fistula tract, insertion of seton suture			
DRH015	n/a	n/a	n/a		Vaginal hysterectomy			

A					1997			
DRH015 B	n/a	n/a	n/a		Vaginal hysterectomy 1998			
DRH015 C	n/a	n/a	n/a		Vaginal hysterectomy 1999			
DRH015 D	n/a	n/a	n/a		Vaginal hysterectomy 2000			
DRH016 A	L1	B1	A3					
DRH016 B	L1	B1	A3					
DRH016 C	L1	B1	A3					
DRH016 D	L1	B1	A3					
DRH017 A	L1+4	B2	A2					
DRH017 B	L1+4	B2	A2					
DRH017 C	L1+4	B2	A2					
DRH017 D	L1+4	B2	A2					
DRH017 E	L1+4	B2	A2					
DRH018 A	L2+4	B2	A2		appendicectomy 2005. gastrojejunostomy 1/11/11	6/9/12	small bowel resection for 4 strictures	
DRH018 B	L2+4	B2	A2					
DRH018 C	L2+4	B2	A2					
DRH018 D	L2+4	B2	A2					

DRH018E	L2+4	B2	A2					
DRH019A	n/a	n/a	n/a			12/10/11	lap to open panproctocolectomy	chronic continuous disease
DRH019B	n/a	n/a	n/a					
DRH020A	L2+4	B2p	A2		subtotal colectomy 30/7/2004, completion proctectomy 8/3/2006, perianal sinus marsupilisationa and negative pressure therapy 1/1/2009			
DRH020B	L2+4	B2p	A2					
DRH020C	L2+4	B2p	A2					
DRH020D	L2+4	B2p	A2					
DRH021A	n/a	n/a	n/a					
DRH021B	n/a	n/a	n/a					
DRH022A	L3	B1	A2		Right hemicolectomy 2007			
DRH022B	L3	B1	A2					
DRH022C	L3	B1	A2					
DRH022D	L3	B1	A2					
DRH023A	n/a	n/a	n/a			26/10/11	colectomy	Acute disease
DRH023	n/a	n/a	n/a					

B								
DRH024A	L2	B1	A2					
DRH024B	L2	B1	A2					
DRH024C	L2	B1	A2					
DRH024D	L2	B1	A2					
DRH024E	L2	B1	A2					
DRH025A	n/a	n/a	n/a					
DRH025B	n/a	n/a	n/a					
DRH025C	n/a	n/a	n/a					
DRH025D	n/a	n/a	n/a					
DRH025E	n/a	n/a	n/a					
DRH026A	L3	B1	A3		appendicectomy 1975			
DRH026B	L3	B1	A3					
DRH026C	L3	B1	A3					
DRH026E	L3	B1	A3					
DRH027A	L3	B1	A2					
DRH027B	L3	B1	A2					
DRH027	L3	B1	A2					

C								
DRH027 D	L3	B1	A2					
DRH028 A	L2	B2	A2		Laparoscopic loop colostomy 30/11/10	7/11/11	panproctocole ctomy	Chronic disease
DRH028 B	L2	B2	A2					
DRH029 A	n/a	n/a	n/a					
DRH029 B	n/a	n/a	n/a					
DRH029 C	n/a	n/a	n/a					
DRH029 D	n/a	n/a	n/a					
DRH030 A	L2	B1	A3					
DRH030 B	L2	B1	A3					
DRH030 C	L2	B1	A3					
DRH030 D	L2	B1	A3					
DRH031 A	L2	B1	A3					
DRH031 B	L2	B1	A3					
DRH031 C	L2	B1	A3					
DRH031 D	L2	B1	A3					
DRH031 E	L2	B1	A3					
DRH032	L2	B1	A3	cervical cancer	hysterectomy 1997,			

A					excision of cervix and pelvic lymphadenectomy for cervical ca 2010, laparoscopic incisional hernia repair 24/8/2011			
DRH032 B	L2	B1	A3					
DRH032 C	L2	B1	A3					
DRH033 A	n/a	n/a	n/a					
DRH033 B	n/a	n/a	n/a					
DRH033 C	n/a	n/a	n/a					
DRH033 D	n/a	n/a	n/a					
DRH034 A	n/a	n/a	n/a					
DRH034 B	n/a	n/a	n/a					
DRH034 C	n/a	n/a	n/a					
DRH034 D	n/a	n/a	n/a					
DRH035 A	L2	B2p	A2		appendicectomy 1970, perianal procedure 1981			
DRH035 B	L2	B2p	A2					
DRH035 C	L2	B2p	A2					
DRH035 D	L2	B2p	A2					
DRH036 A	n/a	n/a	n/a					

DRH036B	n/a	n/a	n/a					
DRH036C	n/a	n/a	n/a					
DRH036D	n/a	n/a	n/a					
DRH037A	n/a	n/a	n/a					
DRH037B	n/a	n/a	n/a					
DRH037C	n/a	n/a	n/a					
DRH037D	n/a	n/a	n/a					
DRH038A	n/a	n/a	n/a					
DRH038B	n/a	n/a	n/a					
DRH038C	n/a	n/a	n/a					
DRH039A	L2	B1	A2		right hemicolectomy 1983, Total Abdominal Hysterectomy for fibroids 9/8/7			
DRH039B	L2	B1	A2					
DRH039C	L2	B1	A2					
DRH039D	L2	B1	A2					
DRH039E	L2	B1	A2					
DRH039F	L2	B1	A2					
DRH040	L3	B2	A1					

A								
DRH040 B	L3	B2	A1		panproctocolectomy 1/1/2004, small bowel resection 1/7/10,	30/11/11	small bowel resection	stricture
DRH041 A	n/a	n/a	n/a					
DRH041 B	n/a	n/a	n/a					
DRH041 C	n/a	n/a	n/a					
DRH041 D	n/a	n/a	n/a					
DRH042 A	n/a	n/a	n/a					
DRH042 B	n/a	n/a	n/a					
DRH042 C	n/a	n/a	n/a					
DRH042 D	n/a	n/a	n/a					
DRH042 E	n/a	n/a	n/a					
DRH042 F	n/a	n/a	n/a			12/12/12	colectomy	continuous chronic disease
DRH043 A	n/a	n/a	n/a	CIN III, dislocated shoulder, UTIs / pyelonephritis, erythema nodosum 07/10	arthroscopy right shoulder 2008, STOP 2001			
DRH043 B	n/a	n/a	n/a					
DRH043 C	n/a	n/a	n/a					
DRH043 D	n/a	n/a	n/a					
DRH044 A	L2+4	B2	A2		small bowel resection 17/3/2009			

DRH044 B	L2+4	B2	A2					
DRH045 A	n/a	n/a	n/a			5/1/12	colectomy	acute on chronic disease
DRH045 B	n/a	n/a	n/a					
DRH046 A	L2	B1	A2					
DRH046 B	L2	B1	A2					
DRH047 A	n/a	n/a	n/a	hypertension				
DRH047 B	n/a	n/a	n/a					
DRH047 C	n/a	n/a	n/a					
DRH047 D	n/a	n/a	n/a					
DRH048 A	L1	B2	A2	asthma, depression	small bowel resection 1/1/2, small bowe resection 1/1/4, small bowel resection 1/1/6, division of adhesions 1/8/10,	17/1/12	strictуroplasty and small bowel resection	strictures
DRH048 B	L1	B2	A2			.		
DRH049 A	L1	B1	A2					
DRH049 B	L1	B1	A2		laparoptomy 7/12/12 looked like small bowel lymphoma	29/1/13	. Laparotomy - excision of SB mass - path showed crohns	Abdo mass
DRH049 C	L1	B1	A2					

DRH049D	L1	B1	A2					
DRH050A	L2	B1	A2					
DRH050B	L2	B1	A2					
DRH051A	L3	B2p	A2		right hemi 1/3/09	19/1/12	Anal dilatation	
DRH051B	L3	B2p	A2					
DRH052A	n/a	n/a	n/a		colectomy 19/5/11 for severe disease	7/2/12	Completion proctectomy and formation of IPAA	
DRH052B	n/a	n/a	n/a					
DRH053A	L1	B1p	A1		EUA 21/2/12, EUA and I&D horseshoe abscess 22/2/12, insertion of seton suture 7/3/12	29/2/12	EUA and I&D and washout perianal abscess, loop ileostomy	Perianal Crohn's with sepsis
DRH053B	L1	B1p	A1					
DRH054A	L2	B2	A3		lap sterilisation 1995	29/2/12	right hemicolectomy	obstructive symptoms
DRH055A	n/a	n/a	n/a			20/3/12	colectomy	severe disease
DRH055B	n/a	n/a	n/a					
DRH056A	n/a	n/a	n/a		colectomy for chronic severe disease 13/4/10	28/3/12	completion proctectomy	elective
DRH057A	L1	B2p	A2		right hemicolectomy 1/1/2000, perianal	30/3/12	small bowel resection	strictures

					abscess with fistula 1/1/01,			
DRH057 B	L1	B2p	A2					
DRH058 A	L2	B1	A3			30/3/12	colectomy	Chronic disease
DRH058 B	L2	B1	A3					
DRH059 A	n/a	n/a	n/a					
DRH059 B	n/a	n/a	n/a					
DRH060 A	n/a	n/a	n/a					
DRH060 B	n/a	n/a	n/a					
DRH063 A	n/a	n/a	n/a					
DRH063 B	n/a	n/a	n/a					
DRH066 A	L3	B3	A2			29/6/12	ileocolic resection and end ileostomy	for CD fistulating into sigmoid
DRH066 B	L3	B3	A2					
DRH067 A	n/a	n/a	n/a			28/6/12	colectomy	severe disease
DRH068 A	n/a	n/a	n/a		Cardiac defect repair as a child			
DRH068 B	n/a	n/a	n/a					
DRH071 A	L3	B1	A2					
DRH071 B	L3	B1	A2					

DRH071C	L3	B1	A2					
DRH072A	L3	B3	A2					
DRH072B	L3	B3	A2					
DRH074A	L1	B3	A3					
DRH075A	L3	B1	A3					
DRH075B	L3	B1	A3					
DRH079A	n/a	n/a	n/a	asthma, dmII, hypothyroid, fibroids, parastomal / incisional herniae, fibromylagia	colectomy 1/1/5	2/7/12	completion proctectomy	elective
DRH079B	n/a	n/a	n/a					
DRH082A						21/8/12	colectomy	severe disease
DRH082B								
DRH083A	L2	B1	A3					
DRH083B	L2	B1	A3					
DRH084A	n/a	n/a	n/a					
DRH084B	n/a	n/a	n/a					
DRH085A	L1	B2	A3			21/9/12	ileocaecal resection with partial cystectomy	enterovesical fistula
DRH085B	L1	B2	A3					

DRH086A	n/a	n/a	n/a					
DRH086B	n/a	n/a	n/a					
DRH090A	L3	B2	A2		appendicectomy 1/1/98 - ? Crohns seen at terminal ileum at time of op but no treatment started			
DRH090B	L3	B2	A2					
DRH091A	n/a	n/a	n/a					
DRH091B	n/a	n/a	n/a					
DRH096A	n/a	n/a	n/a			30/11/12	colectomy	severe disease
DRH096B	n/a	n/a	n/a					
DRH097A	L3	B1	A2		right hemicolectomy 2007, small bowel resection 27/12/10	11/12/12	completion proctectomy	elective
DRH097B	L3	B1	A2					
DRH098A	n/a	n/a	n/a			4/1/13	colectomy	severe disease
DRH098B	n/a	n/a	n/a					
DRH098C	n/a	n/a	n/a					
DRH099A	L3	B3	A2		right colonic resection 1/1/93, sigmoid colectomy, 1/1/93	20/4/12	extended right hemicolectomy, ileostomy and repair of 3 small bowel loops (fistula	

							from transverse colon but SB normal - no evidence of CD)	
DRH099 B	L4	B4	A3					
DRH102 A	L1	B2	A3			13/12/12	small bowel resection for strictures	
DRH102 B	L1	B2	A3					
DRH104 A	L2	B1	A1					
DRH104 B	L2	B1	A1					
DRH105 A	n/a	n/a	n/a					
DRH105 B	n/a	n/a	n/a					
DRH106 A	L1	B2	A2		right hemicolectomy 1/1/5			
DRH106 B	L1	B2	A2					
DRH200 A	L1	B2	A2			23/1/13	ileo-caecal resection (lap assisted)	stricture
DRH200 B	L1	B2	A2					

8.2.2 cont…			
Label	**FHx**	**Notes**	**Date of death**
DRH001A			
DRH001B			
DRH001C			
DRH001D			
DRH001E			
DRH001F			
DRH001G			
DRH002A			
DRH002B			
DRH002C			
DRH002D			
DRH003A	brother (DRH009) sister (DRH006) and father have UC		
DRH003B			
DRH003C			
DRH003D			
DRH004A	father CD	moved from sydney 2010	
DRH004B			
DRH004C			
DRH005A			
DRH005B			
DRH006A	brothers(DRH003+009) and father have UC		
DRH006B			
DRH006C			
DRH006D			
DRH007A			
DRH007B			
DRH007C			
DRH007D			
DRH008A			
DRH008BS			
DRH008BU			
DRH008C			

DRH008D			
DRH009A	brother (DRH003) sister (DRH006) and father have UC	ankle fracture jan12	
DRH009B			
DRH009C			
DRH009D			
DRH010A			
DRH010B			
DRH010C			
DRH010D			
DRH011A			
DRH011B			
DRH011C			
DRH011D			
DRH012A		trial tacrolimus april 2012 - no benefit therefore stopped, pt taking nicotine gum only	
DRH012B			
DRH012C			
DRH012D			
DRH013A			
DRH013B			
DRH013C			
DRH013D			
DRH014A		1st Sample taken pre EUA, fistulotomy and seton insertion	
DRH014B			
DRH014C			
DRH014D			
DRH014E			
DRH015A		2nd samples pre inflix - missed post inflix samples	
DRH015B			
DRH015C			
DRH015D			
DRH016A	cousin colitis	2/4/12 - recent flare requiring pred. finished at time of samples	
DRH016B			

DRH016C			
DRH016D			
DRH017A			
DRH017B			
DRH017C			
DRH017D			
DRH017E			
DRH018A		DRH 018AS blood taken from c-line (no peripheral access)	
DRH018B		DRH018BS blood taken from left radial artery (no peripheral access + c-line not bleeding)	
DRH018C		UGIE 12/10/2011 - duodenal ulceration improving on inflix	
DRH018D		2nd inflix (200mg hydrocortisome before) 19/10/2011 19:00	
DRH018E		01/02/2012 - severe upper gi disease	
DRH019A			
DRH019B			
DRH020A			
DRH020B			
DRH020C			
DRH020D			
DRH021A			
DRH021B		withdrew 16/1/12	
DRH022A		previously had infliximab 2007, stopped methotrexate 09/11, normal colonoscopy 19/10/11	
DRH022B			
DRH022C			
DRH022D			
DRH023A			
DRH023B			
DRH024A		Methotrexate 25mg weekly stopped 20/10/11	
DRH024B			
DRH024C			
DRH024D			
DRH024E			
DRH025A			

DRH025B			
DRH025C			
DRH025D			
DRH025E			
DRH026A			
DRH026B			
DRH026C			
DRH026E			
DRH027A	Non-identical twin (other twin also has CD)		
DRH027B			
DRH027C			
DRH027D			
DRH028A			
DRH028B			
DRH029A	brother has UC and PSC		
DRH029B		flare end of feb requiring 30mg pred 1/52	
DRH029C			
DRH029D		june 12 moved to ada 40mg / wk	
DRH030A	1st cousin CD + cousin's daughter CD		
DRH030B			
DRH030C			
DRH030D			
DRH031A			
DRH031B			
DRH031C			
DRH031D			
DRH031E			
DRH032A			
DRH032B			
DRH032C			
DRH033A			
DRH033B			
DRH033C			
DRH033D			

DRH034A			
DRH034B			
DRH034C			
DRH034D			
DRH035A			
DRH035B			
DRH035C			
DRH035D			
DRH036A			
DRH036B			
DRH036C			
DRH036D			
DRH037A	father CD		
DRH037B			
DRH037C			
DRH037D			
DRH038A			
DRH038B			
DRH038C		? Pregnant, 4th set not taken as confirmed preg	
DRH039A			
DRH039B			
DRH039C			
DRH039D			
DRH039E			
DRH039F			
DRH040A			
DRH040B			
DRH041A			
DRH041B			
DRH041C			
DRH041D			
DRH042A			
DRH042B			
DRH042C			

DRH042D			
DRH042E			
DRH042F			
DRH043A			
DRH043B			
DRH043C			
DRH043D			
DRH044A		viral infection 2007 - unwell after protein losing enetropathy ? Cause. Died 3/2/12	03/02/12
DRH044B		viral infection 2007 - unwell after protein losing enetropathy ? Cause. Died 3/2/12	
DRH045A			
DRH045B			
DRH046A			
DRH046B			
DRH047A			
DRH047B			
DRH047C			
DRH047D			
DRH048A	170cm SB remains as well as large bowel		
DRH048B	170cm SB remains as well as large bowel		
DRH049A			
DRH049B			
DRH049C			
DRH049D			
DRH050A			
DRH050B			
DRH051A			
DRH051B			
DRH052A			
DRH052B			
DRH053A			
DRH053B			
DRH054A			

DRH055A		path indeterminate colitis (clinically more in keeping with UC but path ?CD)	
DRH055B			
DRH056A		post-operatively deranged LFTs. Diagnosed with cholangiocarcinoma. Resection in edinburgh. Chemo. Community arrest - ct showed perforation of intra-abdo viscus with ? Ischaemia of liver, spleen, kidney, pancreas and right kidney with infarcts left kidney.	08/01/13
DRH057A			
DRH057B			
DRH058A			
DRH058B			
DRH059A			
DRH059B		unable to contact after 2nd samples	
DRH060A			
DRH060B			
DRH063A		self catheterises	
DRH063B		self catheterises	
DRH066A			
DRH066B			
DRH067A		PM - generalised acute peritonitis secondary to ileostomy ischaemia	02/08/12
DRH068A			
DRH068B			
DRH071A		4 yrs blood diarrhoea, 4 months erythema nodosum, 4 months oligoarthritis	
DRH071B			
DRH071C			
DRH072A	mother UC, pat aunt CD	stopped pred 40mg / tacro 1mg / mesalazine 800mg bd / fluoxetine 3 weeks ago	
DRH072B	mother UC, pat aunt CD		
DRH074A		follow up colonoscopy - normal bowel ? Findings secondary to severe diverticular disease causing a fistula. Path from colonoscopy biopsies - normal	

DRH075A		admitted with ?obstruction - laparotomy and division of adhesions. Post op pelvic collection - perc drain.	
DRH075B			
DRH079A			
DRH079B			
DRH082A			
DRH082B			
DRH083A			
DRH083B			
DRH084A			
DRH084B			
DRH085A			
DRH085B			
DRH086A			
DRH086B			
DRH090A			
DRH090B			
DRH091A			
DRH091B			
DRH096A			
DRH096B			
DRH097A			
DRH097B			
DRH098A			
DRH098B			
DRH098C			
DRH099A		post op PE	
DRH099B			
DRH102A		previous inflix 04/12 - 10/12 (no response)	
DRH102B			
DRH104A			
DRH104B			
DRH105A			
DRH105B			

DRH106A			
DRH106B			
DRH200A			
DRH200B			

8.3 IBD Patient Sample Collection and Preparation Details						
Biofluid	Sample Date	Sample Label	CollectionTime	SpinTime	FreezeTime	FastedTime
Serum	12-Aug-11	DRH001AS	08:40:00	09:50:00	10:25:00	overnight
Urine	12-Aug-11	DRH001AU	09:10:00	09:35:00	10:25:00	overnight
Serum	17-Aug-11	DRH002AS	09:00:00	10:05:00	10:40:00	overnight
Urine	17-Aug-11	DRH002AU	09:05:00	09:40:00	10:40:00	overnight
Serum	23-Aug-11	DRH003AS	08:40:00	09:50:00	10:20:00	overnight
Urine	23-Aug-11	DRH003AU	08:45:00	09:30:00	10:20:00	overnight
Serum	24-Aug-11	DRH004AS	06:40:00	08:35:00	09:25:00	overnight
Urine	24-Aug-11	DRH004AU	07:20:00	08:25:00	09:25:00	overnight
Serum	24-Aug-11	DRH005AS	10:05:00	11:50:00	12:15:00	overnight
Urine	24-Aug-11	DRH005AU	10:00:00	11:35:00	12:15:00	overnight
Serum	26-Aug-11	DRH006AS	09:50:00	11:10:00	11:35:00	overnight
Urine	26-Aug-11	DRH006AU	09:55:00	10:55:00	11:35:00	overnight
Serum	30-Aug-11	DRH007AS	14:05:00	15:35:00	16:00:00	overnight
Urine	30-Aug-11	DRH007AU	14:10:00	15:20:00	16:00:00	overnight
Serum	31-Aug-11	DRH008AS	08:10:00	10:10:00	10:40:00	overnight
Urine	31-Aug-11	DRH008AU	08:30:00	09:10:00	10:40:00	overnight
Serum	02-Sep-11	DRH009AS	09:05:00	10:25:00	10:50:00	overnight
Urine	02-Sep-11	DRH009AU	09:30:00	10:05:00	10:50:00	overnight
Serum	06-Sep-11	DRH010AS	08:50:00	10:05:00	10:35:00	overnight
Urine	06-Sep-11	DRH010AU	09:00:00	09:40:00	10:35:00	overnight
Serum	08-Sep-11	DRH011AS	10:20:00	12:00:00	13:05:00	overnight
Urine	08-Sep-11	DRH011AU	10:45:00	12:25:00	13:05:00	overnight
Serum	07-Sep-11	DRH005BS	15:05:00	16:10:00	16:50:00	6 hours
Urine	07-Sep-11	DRH005BU	15:10:00	16:00:00	16:50:00	6 hours
Serum	08-Sep-11	DRH012AS	16:40:00	18:35:00	19:05:00	6 hours
Urine	08-Sep-11	DRH012AU	16:50:00	18:25:00	19:05:00	6 hours
Serum	09-Sep-11	DRH013AS	09:15:00	10:35:00	11:10:00	overnight
Urine	09-Sep-11	DRH013AU	09:20:00	10:20:00	11:10:00	overnight

Serum	20-Sep-11	DRH014AS	07:50:00	09:00:00	09:25:00	overnight
Urine	20-Sep-11	DRH014AU	08:00:00	08:35:00	09:25:00	overnight
Serum	27-Sep-11	DRH015AS	10:25:00	12:15:00	12:40:00	overnight
Urine	27-Sep-11	DRH015AU	10:30:00	12:00:00	12:40:00	overnight
Serum	03-Oct-11	DRH016AS	09:15:00	11:05:00	11:35:00	overnight
Urine	03-Oct-11	DRH016AU	09:20:00	10:50:00	11:35:00	overnight
Serum	04-Oct-11	DRH017AS	09:35:00	11:40:00	12:50:00	coffee with milk 09:00
Urine	04-Oct-11	DRH017AU	09:30:00	11:20:00	12:50:00	coffee with milk 09:00
Serum	04-Oct-11	DRH018AS	10:10:00	11:40:00	12:50:00	overnight
Urine	04-Oct-11	DRH018AU	10:15:00	11:20:00	12:50:00	overnight
Serum	06-Oct-11	DRH014BS	10:00:00	11:05:00	11:35:00	overnight
Urine	06-Oct-11	DRH014BU	10:00:00	10:45:00	11:35:00	overnight
Serum	12-Oct-11	DRH019AS	08:20:00	10:05:00	10:30:00	overnight
Urine	12-Oct-11	DRH019AU	08:00:00	08:55:00	10:30:00	overnight
Serum	13-Oct-11	DRH020AS	09:40:00	10:45:00	11:10:00	overnight
Urine	13-Oct-11	DRH020AU	09:45:00	10:35:00	11:10:00	overnight
Serum	18-Oct-11	DRH021AS	07:55:00	09:45:00	10:40:00	overnight
Urine	18-Oct-11	DRH021AU	07:50:00	09:30:00	10:40:00	overnight
Serum	18-Oct-11	DRH017BS	08:45:00	09:45:00	10:40:00	overnight
Urine	18-Oct-11	DRH017BU	08:40:00	09:20:00	10:40:00	overnight
Serum	19-Oct-11	DRH022AS	10:40:00	11:45:00	12:40:00	overnight
Urine	19-Oct-11	DRH022AU	10:45:00	11:00:00	12:40:00	overnight
Serum	20-Oct-11	DRH018BS	08:25:00	09:30:00	09:55:00	overnight
Urine	20-Oct-11	DRH018BU	08:30:00	09:00:00	09:55:00	overnight
Serum	26-Oct-11	DRH023AS	07:40:00	09:05:00	10:30:00	preload 06:00
Urine	26-Oct-11	DRH023AU	07:35:00	08:50:00	10:20:00	preload 06:00
Serum	02-Nov-11	DRH021BS	09:55:00	13:25:00	14:20:00	overnight

Urine	02-Nov-11	DRH021BU	09:50:00	13:10:00	14:20:00	overnight
Serum	02-Nov-11	DRH024AS	11:20:00	13:25:00	14:50:00	tea with milk 07:30
Urine	02-Nov-11	DRH024AU	11:25:00	14:05:00	14:20:00	tea with milk 07:40
Serum	04-Nov-11	DRH025AS	07:20:00	08:20:00	08:50:00	overnight
Urine	04-Nov-11	DRH025AU	07:25:00	08:00:00	08:50:00	overnight
Serum	04-Nov-11	DRH026AS	11:10:00	12:20:00	13:05:00	overnight
Urine	04-Nov-11	DRH026AU	11:15:00	12:10:00	13:05:00	overnight
Serum	07-Nov-11	DRH027AS	09:15:00	10:25:00	11:00:00	overnight
Urine	07-Nov-11	DRH027AU	09:30:00	10:05:00	11:00:00	overnight
Serum	07-Nov-11	DRH028AS	08:00:00	10:25:00	11:00:00	overnight
Urine	07-Nov-11	DRH028AU	08:15:00	09:55:00	11:00:00	overnight
Serum	08-Nov-11	DRH029AS	08:10:00	11:10:00	11:50:00	overnight
Urine	08-Nov-11	DRH029AU	08:15:00	11:00:00	11:50:00	overnight
Serum	08-Nov-11	DRH030AS	09:35:00	11:10:00	11:50:00	overnight
Urine	08-Nov-11	DRH030AU	09:40:00	11:00:00	11:50:00	overnight
Serum	08-Nov-11	DRH031AS	13:15:00	14:30:00	15:55:00	breakfast 06:30, apple 11:00
Urine	08-Nov-11	DRH031AU	13:20:00	14:20:00	15:55:00	breakfast 06:30, apple 11:00
Serum	15-Nov-11	DRH032AS	08:10:00	10:55:00	11:45:00	overnight
Urine	15-Nov-11	DRH032AU	08:15:00	10:20:00	11:45:00	overnight
Serum	15-Nov-11	DRH033AS	09:00:00	10:55:00	11:50:00	overnight
Urine	15-Nov-11	DRH033AU	09:05:00	10:35:00	11:50:00	overnight
Serum	15-Nov-11	DRH001BS	08:45:00	10:55:00	11:45:00	overnight
Urine	15-Nov-11	DRH001BU	08:50:00	10:20:00	11:45:00	overnight
Serum	15-Nov-11	DRH024BS	13:05:00	14:25:00	14:50:00	6 hours
Urine	15-Nov-11	DRH024BU	14:05:00	14:15:00	14:50:00	6 hours
Serum	17-Nov-11	DRH002BS	09:15:00	11:40:00	12:45:00	overnight

Urine	17-Nov-11	DRH002BU	09:20:00	11:30:00	12:45:00	overnight
Serum	17-Nov-11	DRH034AS	09:40:00	11:40:00	12:45:00	overnight
Urine	17-Nov-11	DRH034BS	09:45:00	11:30:00	12:45:00	overnight
Serum	21-Nov-11	DRH035AS	08:40:00	09:50:00	10:30:00	overnight
Urine	21-Nov-11	DRH035AU	08:45:00	09:35:00	10:30:00	overnight
Serum	22-Nov-11	DRH003BS	08:35:00	10:25:00	11:00:00	overnight
Urine	22-Nov-11	DRH003BU	08:40:00	10:15:00	11:00:00	overnight
Serum	22-Nov-11	DRH036AS	09:10:00	10:25:00	11:00:00	overnight
Urine	22-Nov-11	DRH036AU	09:25:00	10:15:00	11:00:00	overnight
Serum	23-Nov-11	DRH037AS	09:05:00	13:20:00	14:20:00	overnight
Urine	23-Nov-11	DRH037AU	09:10:00	12:55:00	14:20:00	overnight
Serum	23-Nov-11	DRH038AS	09:25:00	13:20:00	14:20:00	overnight
Urine	23-Nov-11	DRH038AU	09:30:00	13:10:00	14:20:00	overnight
Serum	23-Nov-11	DRH031BS	10:05:00	13:20:00	14:20:00	overnight
Urine	23-Nov-11	DRH031BU	10:10:00	12:55:00	14:20:00	overnight
Serum	23-Nov-11	DRH008BS	11:00:00	13:20:00	14:20:00	overnight
Serum	25-Nov-11	DRH006BS	09:40:00	11:40:00	12:25:00	overnight
Urine	25-Nov-11	DRH006BU	09:45:00	11:25:00	12:25:00	overnight
Serum	25-Nov-11	DRH011BS	09:00:00	11:40:00	12:25:00	overnight
Urine	25-Nov-11	DRH011BU	09:10:00	11:25:00	12:25:00	overnight
Serum	25-Nov-11	DRH007BS	13:55:00	17:05:00	17:45:00	overnight
Urine	25-Nov-11	DRH007BU	14:05:00	16:55:00	17:25:00	overnight
Serum	25-Nov-11	DRH004BS	15:55:00	17:05:00	17:45:00	overnight
Urine	25-Nov-11	DRH004BU	15:40:00	16:55:00	17:25:00	overnight
Serum	28-Nov-11	DRH039AS	10:10:00	11:40:00	12:05:00	overnight
Urine	28-Nov-11	DRH039AU	10:15:00	11:30:00	12:05:00	overnight
Serum	30-Nov-11	DRH040AS	07:45:00	10:25:00	11:00:00	overnight
Urine	30-Nov-11	DRH040AU	07:50:00	10:10:00	11:00:00	overnight
Serum	02-Dec-11	DRH019BS	10:50:00	11:55:00	12:20:00	overnight
Urine	02-Dec-11	DRH019BU	10:55:00	11:40:00	12:20:00	overnight
Serum	07-Dec-11	DRH041AS	09:10:00	10:30:00	11:20:00	overnight

Urine	07-Dec-11	DRH041AU	09:15:00	10:45:00	11:10:00	overnight
Serum	08-Dec-11	DRH009BS	09:05:00	10:30:00	11:00:00	overnight
Urine	08-Dec-11	DRH009BU	09:10:00	10:10:00	11:00:00	overnight
Serum	12-Dec-11	DRH014CS	08:55:00	10:30:00	11:00:00	overnight
Urine	12-Dec-11	DRH014CU	09:00:00	10:10:00	11:00:00	overnight
Serum	12-Dec-11	DRH042AS	09:15:00	10:30:00	11:00:00	overnight
Urine	12-Dec-11	DRH042AU	09:20:00	10:20:00	11:00:00	overnight
Serum	14-Dec-11	DRH043AS	09:10:00	12:05:00	12:45:00	overnight
Urine	14-Dec-11	DRH043AU	09:15:00	11:40:00	12:45:00	overnight
Serum	14-Dec-11	DRH015BS	10:05:00	12:05:00	13:45:00	cereal at 08:00
Urine	14-Dec-11	DRH015BU	10:10:00	11:40:00	13:45:00	cereal at 08:00
Serum	14-Dec-11	DRH012BS	10:25:00	12:05:00	12:45:00	overnight
Urine	14-Dec-11	DRH012BU	10:30:00	11:50:00	12:45:00	overnight
Urine	14-Dec-11	DRH008BU	16:20:00	17:20:00	18:35:00	6 hours
Serum	15-Dec-11	DRH044AS	11:20:00	13:25:00	13:45:00	overnight
Urine	15-Dec-11	DRH044AU	12:10:00	13:10:00	13:45:00	overnight
Serum	04-Jan-12	DRH044BS	10:00:00	11:35:00	12:00:00	overnight
Serum	05-Jan-12	DRH045AS	07:00:00	10:10:00	11:05:00	overnight
Urine	05-Jan-12	DRH045AU	07:10:00	10:25:00	11:05:00	overnight
Serum	08-Jan-12	DRH046AS	13:35:00	14:45:00	15:55:00	overnight
Urine	08-Jan-12	DRH046AU	13:50:00	14:30:00	15:55:00	overnight
Serum	09-Jan-12	DRH016BS	08:50:00	10:25:00	10:50:00	overnight
Urine	09-Jan-12	DRH016BU	08:55:00	09:40:00	09:55:00	overnight
Serum	10-Jan-12	DRH010BS	09:10:00	11:10:00	11:50:00	overnight
Urine	10-Jan-12	DRH010BU	09:10:00	10:45:00	11:50:00	overnight
Serum	10-Jan-12	DRH047AS	09:25:00	11:10:00	11:50:00	overnight
Urine	10-Jan-12	DRH047AU	09:30:00	10:45:00	11:50:00	overnight
Serum	10-Jan-12	DRH020BS	09:35:00	11:10:00	11:50:00	overnight
Urine	10-Jan-12	DRH020BU	09:40:00	11:00:00	11:50:00	overnight
Serum	11-Jan-12	DRH017CS	09:00:00	10:50:00	12:05:00	overnight

Urine	11-Jan-12	DRH017CU	08:55:00	10:40:00	12:05:00	overnight
Serum	17-Jan-12	DRH048AS	08:35:00	10:10:00	10:40:00	overnight
Urine	17-Jan-12	DRH048AU	08:45:00	10:00:00	10:40:00	overnight
Serum	17-Jan-12	DRH049AS	14:05:00	15:10:00	15:35:00	overnight
Urine	17-Jan-12	DRH049AU	14:10:00	14:40:00	15:35:00	overnight
Serum	18-Jan-12	DRH050AS	09:40:00	12:00:00	12:40:00	overnight
Urine	18-Jan-12	DRH050AU	09:35:00	11:50:00	12:40:00	overnight
Serum	18-Jan-12	DRH013BS	10:30:00	12:00:00	12:40:00	overnight
Urine	18-Jan-12	DRH013BU	10:40:00	11:50:00	12:40:00	overnight
Serum	19-Jan-12	DRH051AS	12:35:00	14:00:00	14:25:00	6 hours
Urine	19-Jan-12	DRH051AU	12:40:00	13:50:00	14:25:00	6 hours
Serum	23-Jan-12	DRH022BS	09:05:00	10:45:00	11:10:00	overnight
Urine	23-Jan-12	DRH022BU	09:10:00	10:35:00	11:10:00	overnight
Serum	27-Jan-12	DRH025BS	07:20:00	08:30:00	08:55:00	overnight
Urine	27-Jan-12	DRH025BU	07:25:00	08:20:00	08:55:00	overnight
Serum	27-Jan-12	DRH026BS	11:05:00	12:20:00	13:00:00	overnight
Urine	27-Jan-12	DRH026BU	11:10:00	12:10:00	13:00:00	overnight
Serum	30-Jan-12	DRH027BS	09:45:00	10:50:00	11:15:00	overnight
Urine	30-Jan-12	DRH027BU	09:50:00	10:40:00	11:15:00	overnight
Serum	07-Feb-12	DRH052AS	08:10:00	09:30:00	10:25:00	overnight
Urine	07-Feb-12	DRH052AU	08:15:00	09:20:00	10:25:00	overnight
Serum	31-Jan-12	DRH049BS	14:15:00	15:25:00	16:10:00	6 hours
Urine	31-Jan-12	DRH049BU	14:15:00	15:00:00	15:55:00	6 hours
Serum	01-Feb-12	DRH018CS	09:40:00	11:40:00	12:10:00	overnight
Urine	01-Feb-12	DRH018CU	09:10:00	11:00:00	12:10:00	overnight
Serum	01-Feb-12	DRH050BS	10:10:00	11:40:00	12:10:00	overnight
Urine	01-Feb-12	DRH050BU	10:00:00	11:00:00	12:10:00	overnight
Serum	02-Feb-12	DRH046BS	09:55:00	11:05:00	11:30:00	overnight
Urine	02-Feb-12	DRH046BU	09:40:00	10:10:00	11:30:00	overnight
Serum	08-Feb-12	DRH001CS	08:55:00	11:20:00	12:00:00	overnight
Urine	08-Feb-12	DRH001CU	09:05:00	10:50:00	12:00:00	overnight

Serum	08-Feb-12	DRH024CS	09:15:00	11:20:00	12:00:00	overnight
Urine	08-Feb-12	DRH024CU	09:55:00	11:00:00	12:00:00	overnight
Serum	08-Feb-12	DRH030BS	09:30:00	11:35:00	12:00:00	overnight
Urine	08-Feb-12	DRH030BU	09:35:00	10:50:00	12:00:00	overnight
Serum	13-Feb-12	DRH034BS	14:55:00	16:20:00	16:45:00	7.5 hours
Urine	13-Feb-12	DRH034BU	15:00:00	16:10:00	16:45:00	7.5 hours
Serum	14-Feb-12	DRH032BS	08:00:00	09:55:00	10:25:00	overnight
Urine	14-Feb-12	DRH032BU	08:05:00	09:45:00	10:25:00	overnight
Serum	14-Feb-12	DRH037BS	08:45:00	09:55:00	10:25:00	overnight
Urine	14-Feb-12	DRH037BU	08:50:00	09:45:00	10:25:00	overnight
Serum	15-Feb-12	DRH011CS	10:05:00	12:00:00	12:25:00	overnight
Urine	15-Feb-12	DRH011CU	10:05:00	11:50:00	12:25:00	overnight
Serum	16-Feb-12	DRH035BS	09:00:00	10:15:00	10:50:00	overnight
Urine	16-Feb-12	DRH035BU	09:20:00	10:30:00	10:50:00	overnight
Serum	17-Feb-12	DRH002CS	09:05:00	10:25:00	10:55:00	overnight
Urine	17-Feb-12	DRH002CU	09:10:00	10:15:00	10:55:00	overnight
Serum	20-Feb-12	DRH031CS	09:25:00	10:30:00	11:25:00	overnight
Urine	20-Feb-12	DRH031CU	09:30:00	09:55:00	11:25:00	overnight
Serum	20-Feb-12	DRH038BS	08:25:00	10:05:00	11:25:00	overnight
Urine	20-Feb-12	DRH038BU	08:30:00	09:55:00	11:25:00	overnight
Serum	21-Feb-12	DRH033BS	09:05:00	10:55:00	11:30:00	overnight
Urine	21-Feb-12	DRH033BU	09:10:00	10:45:00	11:30:00	overnight
Serum	22-Feb-12	DRH001DS	09:10:00	10:20:00	11:00:00	overnight
Urine	22-Feb-12	DRH001DU	09:00:00	10:10:00	11:00:00	overnight
Serum	22-Feb-12	DRH025CS	08:50:00	10:20:00	11:00:00	overnight
Urine	22-Feb-12	DRH025CU	08:40:00	10:10:00	11:00:00	overnight
Serum	24-Feb-12	DRH007CS	14:05:00	15:15:00	15:40:00	overnight
Urine	24-Feb-12	DRH007CU	14:10:00	14:50:00	15:40:00	overnight
Serum	27-Feb-12	DRH003CS	09:05:00	10:10:00	10:30:00	overnight
Urine	27-Feb-12	DRH003CU	09:05:00	10:00:00	10:30:00	overnight
Serum	28-Feb-12	DRH036BS	09:25:00	13:00:00	13:30:00	overnight

Urine	28-Feb-12	DRH036BU	09:30:00	12:50:00	13:30:00	overnight
Serum	28-Feb-12	DRH039BS	11:10:00	13:00:00	13:30:00	overnight
Urine	28-Feb-12	DRH039BU	11:15:00	12:50:00	13:30:00	overnight
Serum	29-Feb-12	DRH053AS	07:10:00	10:15:00	11:30:00	overnight
Urine	29-Feb-12	DRH053AU	07:00:00	10:00:00	11:30:00	overnight
Serum	29-Feb-12	DRH054AS	07:30:00	10:15:00	11:30:00	overnight
Urine	29-Feb-12	DRH054AU	07:25:00	10:00:00	11:30:00	overnight
Serum	01-Mar-12	DRH029BS	08:55:00	10:15:00	10:40:00	overnight
Urine	01-Mar-12	DRH029BU	09:00:00	10:00:00	10:40:00	overnight
Serum	02-Mar-12	DRH006CS	09:20:00	12:35:00	13:10:00	overnight
Urine	02-Mar-12	DRH006CU	09:25:00	12:25:00	13:10:00	overnight
Serum	02-Mar-12	DRH028BS	10:25:00	13:20:00	13:50:00	overnight
Urine	02-Mar-12	DRH028BU	10:20:00	12:55:00	13:50:00	overnight
Serum	02-Mar-12	DRH040BS	11:45:00	13:20:00	13:50:00	overnight
Urine	02-Mar-12	DRH040BU	11:40:00	12:55:00	13:50:00	overnight
Serum	02-Mar-12	DRH045BS	09:50:00	12:35:00	13:10:00	overnight
Urine	02-Mar-12	DRH045BU	09:00:00	12:25:00	13:10:00	overnight
Serum	07-Mar-12	DRH025DS	08:45:00	10:30:00	10:55:00	overnight
Urine	07-Mar-12	DRH025DU	08:40:00	10:20:00	10:55:00	overnight
Serum	08-Mar-12	DRH009CS	08:40:00	10:25:00	10:50:00	overnight
Urine	08-Mar-12	DRH009CU	08:40:00	10:15:00	10:50:00	overnight
Serum	08-Mar-12	DRH042BS	09:05:00	10:25:00	10:50:00	overnight
Urine	08-Mar-12	DRH042BU	09:10:00	10:15:00	10:50:00	overnight
Serum	10-Mar-12	DRH023BS	09:05:00	10:30:00	11:00:00	overnight
Urine	10-Mar-12	DRH023BU	09:20:00	10:10:00	11:00:00	overnight
Serum	13-Mar-12	DRH041BS	09:10:00	10:15:00	10:40:00	overnight
Urine	13-Mar-12	DRH041BU	09:15:00	10:05:00	10:40:00	overnight
Serum	14-Mar-12	DRH014DS	09:15:00	11:15:00	11:50:00	overnight
Urine	14-Mar-12	DRH014DU	08:55:00	10:50:00	11:50:00	overnight
Serum	14-Mar-12	DRH015CS	10:15:00	11:15:00	11:50:00	overnight
Urine	14-Mar-12	DRH015CU	10:20:00	10:50:00	11:50:00	overnight

Serum	20-Mar-12	DRH055AS	09:10:00	12:10:00	12:40:00	overnight
Urine	20-Mar-12	DRH055AU	09:15:00	12:00:00	12:40:00	overnight
Serum	22-Mar-12	DRH012CS	14:30:00	15:40:00	16:00:00	6 hours
Urine	22-Mar-12	DRH012CU	14:20:00	15:30:00	16:00:00	6 hours
Serum	23-Mar-12	DRH051BS	12:20:00	13:55:00	14:15:00	6 hours
Urine	23-Mar-12	DRH051BU	12:15:00	13:45:00	14:15:00	6 hours
Serum	27-Mar-12	DRH010CS	09:15:00	10:20:00	11:45:00	overnight
Urine	27-Mar-12	DRH010CU	09:20:00	10:10:00	11:45:00	overnight
Serum	28-Mar-12	DRH008CS	09:00:00	10:10:00	11:50:00	overnight
Urine	28-Mar-12	DRH008CU	09:05:00	10:00:00	11:50:00	overnight
Serum	28-Mar-12	DRH056AS	05:50:00	08:35:00	09:15:00	overnight
Urine	28-Mar-12	DRH056AU	05:55:00	08:20:00	09:15:00	overnight
Serum	29-Mar-12	DRH058AS	15:00:00	16:05:00	16:30:00	6 hours
Urine	30-Mar-12	DRH058AU	08:55:00	11:20:00	12:30:00	overnight
Serum	30-Mar-12	DRH057AS	08:10:00	11:55:00	12:30:00	overnight
Urine	30-Mar-12	DRH057AU	08:30:00	11:20:00	12:30:00	overnight
Serum	30-Mar-12	DRH020CS	09:30:00	11:55:00	12:30:00	overnight
Urine	30-Mar-12	DRH020CU	09:35:00	11:30:00	12:30:00	overnight
Serum	02-Apr-12	DRH016CS	09:05:00	10:10:00	10:30:00	overnight
Urine	02-Apr-12	DRH016CU	09:10:00	09:40:00	10:30:00	overnight
Serum	03-Apr-12	DRH017DS	08:45:00	09:50:00	10:40:00	overnight
Urine	03-Apr-12	DRH017DU	08:40:00	09:40:00	10:40:00	overnight
Serum	04-Apr-12	DRH053BS	15:55:00	16:55:00	17:15:00	6 hours
Urine	04-Apr-12	DRH053BU	15:50:00	16:20:00	17:15:00	6 hours
Serum	20-Apr-12	DRH099AS	12:30:00	14:40:00	14:50:00	6 hours
Urine	20-Apr-12	DRH099AU	12:20:00	14:30:00	15:10:00	6 hours
Serum	05-Apr-12	DRH048BS	16:10:00	17:15:00	17:45:00	6 hours
Urine	05-Apr-12	DRH048BU	16:50:00	17:15:00	17:30:00	6 hours
Serum	26-Apr-12	DRH070S	13:10:00	14:15:00	14:45:00	overnight
Urine	26-Apr-12	DRH070U	13:10:00	13:55:00	14:45:00	overnight
Serum	16-Apr-12	DRH004CS	15:00:00	16:10:00	16:45:00	6 hours

Urine	16-Apr-12	DRH004CU	14:40:00	15:50:00	16:45:00	6 hours
Serum	02-May-12	DRH052BS	06:35:00	11:00:00	11:45:00	overnight
Urine	02-May-12	DRH052BU	06:40:00	10:45:00	11:45:00	overnight
Serum	02-May-12	DRH026CS	10:05:00	11:20:00	11:45:00	overnight
Urine	02-May-12	DRH026CU	10:15:00	10:45:00	11:45:00	overnight
Serum	08-May-12	DRH030CS	09:45:00	10:50:00	11:15:00	overnight
Urine	08-May-12	DRH030CU	09:40:00	10:20:00	11:15:00	overnight
Serum	08-May-12	DRH047BS	09:00:00	10:30:00	11:15:00	overnight
Urine	08-May-12	DRH047BU	09:05:00	10:20:00	11:15:00	overnight
Serum	08-May-12	DRH043BS	11:45:00	12:45:00	13:10:00	overnight
Urine	08-May-12	DRH043BU	11:50:00	12:25:00	13:10:00	overnight
Serum	09-May-12	DRH027CS	10:00:00	11:10:00	11:35:00	overnight
Urine	09-May-12	DRH027CU	10:05:00	11:00:00	11:35:00	overnight
Serum	10-May-12	DRH001ES	08:35:00	11:10:00	11:35:00	overnight
Serum	11-May-12	DRH059AS	08:00:00	09:40:00	10:00:00	just ate roll / jam / coffee
Urine	11-May-12	DRH059AU	07:50:00	09:30:00	10:00:00	overnight
Serum	12-May-12	DRH055BS	11:40:00	13:00:00	13:25:00	overnight
Urine	12-May-12	DRH055BU	12:35:00	12:45:00	13:25:00	overnight
Serum	14-May-12	DRH034CS	09:00:00	10:55:00	12:00:00	overnight
Urine	14-May-12	DRH034CU	09:05:00	11:40:00	12:00:00	overnight
Serum	15-May-12	DRH037CS	09:00:00	11:05:00	11:25:00	overnight
Urine	15-May-12	DRH037CU	09:05:00	10:55:00	11:25:00	overnight
Serum	17-May-12	DRH032CS	08:00:00	09:35:00	10:15:00	overnight
Urine	17-May-12	DRH032CU	07:55:00	09:25:00	10:15:00	overnight
Serum	18-May-12	DRH035CS	08:55:00	10:50:00	11:15:00	overnight
Urine	18-May-12	DRH035CU	09:00:00	10:40:00	11:15:00	overnight
Serum	23-May-12	DRH060AS	09:30:00	10:35:00	11:00:00	overnight
Urine	23-May-12	DRH060AU	09:15:00	10:20:00	11:00:00	overnight
Serum	24-May-12	DRH002DS	09:15:00	10:25:00	10:50:00	overnight

Urine	24-May-12	DRH002DU	09:20:00	10:15:00	10:50:00	overnight
Serum	25-May-12	DRH038CS	08:35:00	10:05:00	10:25:00	overnight
Urine	25-May-12	DRH038CU	08:40:00	09:55:00	10:25:00	overnight
Serum	25-May-12	DRH057BS	11:55:00	13:05:00	13:35:00	6 hours
Urine	25-May-12	DRH057BU	12:00:00	12:55:00	13:35:00	6 hours
Serum	25-May-12	DRH007DS	13:55:00	15:55:00	16:25:00	overnight
Urine	25-May-12	DRH007DU	14:00:00	15:45:00	16:25:00	overnight
Serum	28-May-12	DRH036CS	08:55:00	10:20:00	10:45:00	overnight
Urine	28-May-12	DRH036CU	09:00:00	10:10:00	10:45:00	overnight
Serum	29-May-12	DRH003DS	08:55:00	12:30:00	13:00:00	overnight
Urine	29-May-12	DRH003DU	09:00:00	12:20:00	13:00:00	overnight
Serum	29-May-12	DRH061S	08:35:00	12:30:00	13:00:00	overnight
Urine	29-May-12	DRH061U	08:55:00	12:20:00	13:00:00	overnight
Serum	30-May-12	DRH025ES	08:55:00	11:05:00	11:50:00	overnight
Urine	30-May-12	DRH025EU	08:45:00	10:55:00	11:50:00	overnight
Serum	30-May-12	DRH024DS	10:20:00	11:20:00	11:50:00	overnight
Urine	30-May-12	DRH024DU	10:10:00	10:55:00	11:50:00	overnight
Serum	31-May-12	DRH022CS	11:40:00	12:45:00	13:10:00	overnight
Urine	31-May-12	DRH022CU	11:45:00	12:20:00	13:10:00	overnight
Serum	01-Jun-12	DRH006DS	08:40:00	10:00:00	10:20:00	overnight
Urine	01-Jun-12	DRH006DU	08:45:00	09:50:00	10:20:00	overnight
Serum	05-Jun-12	DRH033CS	08:50:00	10:55:00	11:15:00	overnight
Urine	05-Jun-12	DRH033CU	08:55:00	10:45:00	11:15:00	overnight
Serum	05-Jun-12	DRH031DS	11:45:00	12:55:00	13:20:00	6 hours
Urine	05-Jun-12	DRH031DU	11:50:00	12:45:00	13:20:00	6 hours
Serum	26-May-12	DRH062S	08:30:00	09:30:00	09:55:00	overnight
Urine	26-May-12	DRH062U	08:40:00	09:10:00	09:55:00	overnight
Serum	06-Jun-12	DRH063AS	09:15:00	10:25:00	11:05:00	overnight
Urine	06-Jun-12	DRH063AU	09:00:00	10:15:00	11:05:00	overnight
Serum	07-Jun-12	DRH009DS	09:00:00	10:55:00	11:40:00	overnight
Urine	07-Jun-12	DRH009DU	09:05:00	09:55:00	11:40:00	overnight

Serum	07-Jun-12	DRH060BS	09:25:00	10:55:00	11:40:00	overnight
Urine	07-Jun-12	DRH060BU	09:15:00	09:55:00	11:40:00	overnight
Serum	07-Jun-12	DRH064S	12:45:00	15:10:00	15:35:00	6 hours
Urine	07-Jun-12	DRH064U	13:50:00	14:55:00	15:35:00	6 hours
Serum	06-Jun-12	DRH011DS	13:30:00	14:35:00	15:15:00	6 hours
Urine	06-Jun-12	DRH011DU	13:25:00	14:00:00	15:15:00	6 hours
Serum	08-Jun-12	DRH042CS	09:00:00	10:50:00	11:15:00	overnight
Urine	08-Jun-12	DRH042CU	09:05:00	10:40:00	11:15:00	overnight
Serum	08-Jun-12	DRH065S	13:10:00	14:15:00	14:40:00	6 hours
Urine	08-Jun-12	DRH065U	13:05:00	13:45:00	14:40:00	6 hours
Serum	15-Jun-12	DRH058BS	10:00:00	10:50:00	11:40:00	overnight
Urine	15-Jun-12	DRH058BU	09:55:00	11:05:00	11:40:00	overnight
Serum	19-Jun-12	DRH001FS	09:15:00	14:55:00	15:15:00	preload 06:15
Urine	19-Jun-12	DRH001FU	09:40:00	14:45:00	15:15:00	preload 06:15
Serum	21-Jun-12	DRH015DS	10:15:00	12:50:00	13:15:00	overnight
Urine	21-Jun-12	DRH015DU	10:20:00	12:40:00	13:15:00	overnight
Serum	22-Jun-12	DRH010DS	10:20:00	11:30:00	11:50:00	overnight
Urine	22-Jun-12	DRH010DU	10:25:00	11:20:00	11:50:00	overnight
Serum	27-Jun-12	DRH066AS	06:30:00	10:45:00	11:10:00	overnight
Urine	27-Jun-12	DRH066AU	06:35:00	10:30:00	11:10:00	overnight
Serum	28-Jun-12	DRH067AS	08:05:00	09:30:00	09:50:00	overnight
Urine	28-Jun-12	DRH067AU	08:10:00	09:20:00	09:50:00	overnight
Serum	28-Jun-12	DRH039CS	15:05:00	17:00:00	17:25:00	6 hours
Urine	28-Jun-12	DRH039CU	15:10:00	16:50:00	17:25:00	6 hours
Serum	29-Jun-12	DRH029CS	09:05:00	10:35:00	11:00:00	overnight
Urine	29-Jun-12	DRH029CU	09:10:00	10:25:00	11:00:00	overnight
Serum	02-Jul-12	DRH079AS	06:40:00	09:30:00	10:20:00	overnight
Urine	02-Jul-12	DRH079AU	06:10:00	09:50:00	10:20:00	overnight
Urine	05-Jul-12	DRH068AU	07:00:00	09:00:00	09:45:00	overnight
Serum	05-Jul-12	DRH068AS	07:00:00	09:10:00	09:45:00	overnight

Serum	05-Jul-12	DRH014ES	10:55:00	12:20:00	12:45:00	overnight
Urine	05-Jul-12	DRH014EU	11:00:00	12:10:00	12:45:00	overnight
Serum	06-Jul-12	DRH013CS	10:45:00	12:10:00	13:05:00	overnight
Urine	06-Jul-12	DRH013CU	10:50:00	11:40:00	13:05:00	overnight
Serum	06-Jul-12	DRH020DS	09:15:00	12:10:00	13:05:00	overnight
Urine	06-Jul-12	DRH020DU	09:20:00	11:30:00	13:05:00	overnight
Serum	06-Jul-12	DRH041CS	10:45:00	12:10:00	13:15:00	overnight
Urine	06-Jul-12	DRH041CU	10:50:00	11:40:00	13:05:00	overnight
Serum	06-Jul-12	DRH069S	10:45:00	12:10:00	13:15:00	overnight
Urine	06-Jul-12	DRH069U	10:40:00	11:55:00	13:05:00	overnight
Serum	20-Jun-12	DRH008DS	15:15:00	16:25:00	16:50:00	6 hours
Urine	20-Jun-12	DRH008DU	16:00:00	16:05:00	16:50:00	6 hours
Serum	20-Jun-12	DRH063BS	09:05:00	10:15:00	11:00:00	overnight
Urine	20-Jun-12	DRH063BU	09:15:00	09:35:00	11:00:00	overnight
Serum	09-Jul-12	DRH016DS	09:10:00	11:55:00	12:15:00	overnight
Urine	09-Jul-12	DRH016DU	09:20:00	11:40:00	12:15:00	overnight
Serum	17-Jul-12	DRH071AS	09:05:00	11:05:00	11:30:00	overnight
Urine	17-Jul-12	DRH071AU	09:10:00	10:55:00	11:30:00	overnight
Serum	18-Jul-12	DRH068BS	10:10:00	11:15:00	11:40:00	overnight
Urine	18-Jul-12	DRH068BU	10:00:00	10:25:00	11:40:00	overnight
Serum	19-Jul-12	DRH072AS	06:05:00	07:10:00	07:35:00	overnight
Urine	19-Jul-12	DRH072AU	06:10:00	06:50:00	07:35:00	overnight
Serum	19-Jul-12	DRH073S	12:55:00	14:30:00	14:50:00	6 hours
Serum	14-Aug-12	DRH037DS	09:10:00	10:50:00	11:20:00	overnight
Urine	14-Aug-12	DRH037DU	09:15:00	10:40:00	11:20:00	overnight
Serum	14-Aug-12	DRH012DS	13:10:00	14:50:00	15:35:00	6 hours
Urine	14-Aug-12	DRH012DU	13:15:00	14:40:00	15:35:00	6 hours
Serum	14-Aug-12	DRH074AS	12:30:00	14:50:00	15:35:00	overnight
Urine	14-Aug-12	DRH074AU	12:35:00	14:40:00	15:35:00	overnight
Serum	15-Aug-12	DRH026DS	10:10:00	11:35:00	12:00:00	overnight
Urine	15-Aug-12	DRH026DU	10:15:00	11:10:00	12:00:00	overnight

Serum	15-Aug-12	DRH075AS	06:35:00	10:55:00	12:00:00	overnight
Urine	15-Aug-12	DRH075AU	06:40:00	10:40:00	12:00:00	overnight
Serum	17-Aug-12	DRH076S	08:25:00	11:25:00	12:35:00	overnight
Urine	17-Aug-12	DRH076U	08:20:00	11:15:00	12:35:00	overnight
Serum	17-Aug-12	DRH077S	08:50:00	11:25:00	12:35:00	overnight
Urine	17-Aug-12	DRH077U	08:35:00	11:15:00	12:35:00	overnight
Serum	17-Aug-12	DRH080S	10:55:00	12:10:00	12:35:00	overnight
Urine	17-Aug-12	DRH080U	10:50:00	12:00:00	12:35:00	overnight
Serum	17-Aug-12	DRH078S	13:10:00	16:20:00	16:50:00	6 hours
Urine	17-Aug-12	DRH078U	13:50:00	16:05:00	16:50:00	6 hours
Serum	17-Aug-12	DRH081S	12:45:00	16:20:00	16:50:00	overnight
Urine	17-Aug-12	DRH081U	12:50:00	15:55:00	16:50:00	overnight
Serum	20-Aug-12	DRH047CS	09:05:00	11:10:00	11:40:00	overnight
Serum	20-Aug-12	DRH030DS	09:35:00	11:10:00	11:40:00	overnight
Urine	20-Aug-12	DRH030DU	09:40:00	11:00:00	11:40:00	overnight
Serum	21-Aug-12	DRH082AS	05:50:00	08:50:00	09:20:00	overnight
Urine	21-Aug-12	DRH082AU	05:55:00	08:40:00	09:20:00	overnight
Serum	22-Aug-12	DRH017ES	09:10:00	10:25:00	11:00:00	overnight
Urine	22-Aug-12	DRH017EU	09:05:00	10:40:00	11:00:00	overnight
Serum	28-Aug-12	DRH033DS	08:00:00	09:05:00	09:30:00	overnight
Urine	28-Aug-12	DRH033DU	08:05:00	08:45:00	09:30:00	overnight
Urine	20-Aug-12	DRH047CU	10:52:00	15:52:00	17:05:00	overnight
Serum	28-Aug-12	DRH083AS	14:00:00	15:35:00	16:00:00	6 hours
Urine	28-Aug-12	DRH083AU	13:55:00	15:25:00	16:00:00	6 hours
Serum	31-Aug-12	DRH034DS	09:05:00	13:35:00	14:30:00	overnight
Urine	31-Aug-12	DRH034DU	09:10:00	13:25:00	14:30:00	overnight
Serum	31-Aug-12	DRH001GS	13:00:00	14:05:00	14:30:00	overnight
Urine	31-Aug-12	DRH001GU	11:25:00	13:25:00	14:30:00	overnight
Serum	04-Sep-12	DRH099BS	09:05:00	11:55:00	12:20:00	overnight
Urine	04-Sep-12	DRH099BU	09:10:00	11:40:00	12:20:00	overnight
Serum	05-Sep-12	DRH036DS	09:15:00	10:55:00	11:35:00	overnight

Urine	05-Sep-12	DRH036DU	09:20:00	10:45:00	11:35:00	overnight
Serum	06-Sep-12	DRH018DS	10:35:00	12:25:00	14:35:00	overnight
Urine	06-Sep-12	DRH018DU	10:35:00	12:00:00	12:40:00	overnight
Serum	06-Sep-12	DRH035DS	08:30:00	12:10:00	12:40:00	overnight
Urine	06-Sep-12	DRH035DU	08:35:00	12:00:00	12:40:00	overnight
Serum	12-Sep-12	DRH083BS	14:55:00	16:20:00	16:45:00	6 hours
Urine	12-Sep-12	DRH083BU	15:00:00	16:10:00	16:45:00	6 hours
Serum	14-Sep-12	DRH079BS	12:50:00	14:20:00	14:40:00	6 hours
Urine	14-Sep-12	DRH079BU	13:00:00	14:10:00	14:40:00	6 hours
Serum	17-Sep-12	DRH066BS	14:40:00	15:45:00	16:20:00	6 hours
Urine	17-Sep-12	DRH066BU	14:45:00	15:15:00	16:20:00	6 hours
Serum	19-Sep-12	DRH024ES	09:00:00	11:25:00	12:25:00	overnight
Urine	19-Sep-12	DRH024EU	09:05:00	11:10:00	12:25:00	overnight
Serum	20-Sep-12	DRH084AS	09:05:00	11:25:00	12:00:00	overnight
Urine	20-Sep-12	DRH084AU	09:00:00	11:15:00	12:00:00	overnight
Serum	21-Sep-12	DRH085AS	08:20:00	11:45:00	12:15:00	overnight
Urine	21-Sep-12	DRH085AU	08:15:00	11:35:00	12:15:00	overnight
Serum	21-Sep-12	DRH027DS	10:05:00	12:15:00	12:45:00	overnight
Urine	21-Sep-12	DRH027DU	10:20:00	12:05:00	12:45:00	overnight
Serum	21-Sep-12	DRH042DS	09:10:00	12:15:00	12:45:00	overnight
Urine	21-Sep-12	DRH042DU	09:15:00	12:05:00	12:45:00	overnight
Serum	01-Oct-12	DRH071BS	14:40:00	15:50:00	16:15:00	6 hours
Urine	01-Oct-12	DRH071BU	14:45:00	15:40:00	16:15:00	6 hours
Serum	02-Oct-12	DRH022DS	09:00:00	10:30:00	11:00:00	overnight
Urine	02-Oct-12	DRH022DU	09:05:00	10:20:00	11:00:00	overnight
Serum	02-Oct-12	DRH031ES	11:55:00	13:25:00	13:55:00	6 hours
Urine	02-Oct-12	DRH031EU	11:50:00	13:10:00	13:55:00	6 hours
Serum	04-Oct-12	DRH084BS	09:00:00	11:30:00	12:10:00	overnight
Urine	04-Oct-12	DRH084BU	08:55:00	11:20:00	12:10:00	overnight
Serum	15-Oct-12	DRH013DS	08:55:00	10:15:00	10:45:00	overnight
Urine	15-Oct-12	DRH013DU	09:00:00	10:05:00	10:45:00	overnight

Serum	09-Oct-12	DRH039DS	09:30:00	10:40:00	11:00:00	overnight
Urine	09-Oct-12	DRH039DU	08:55:00	09:55:00	11:00:00	overnight
Serum	23-Oct-12	DRH043CS	12:20:00	13:30:00	13:55:00	overnight
Urine	23-Oct-12	DRH043CU	12:25:00	13:15:00	13:55:00	overnight
Serum	25-Oct-12	DRH041DS	09:05:00	10:40:00	11:05:00	overnight
Urine	25-Oct-12	DRH041DU	09:10:00	10:30:00	11:05:00	overnight
Serum	26-Oct-12	DRH059BS	12:55:00	14:25:00	14:50:00	6 hours
Serum	29-Oct-12	DRH082BS	14:00:00	15:05:00	15:30:00	6 hours
Urine	29-Oct-12	DRH082BU	14:05:00	14:55:00	15:30:00	6 hours
Serum	31-Oct-12	DRH086AS	06:05:00	08:40:00	09:25:00	overnight
Urine	31-Oct-12	DRH086AU	06:30:00	08:30:00	09:25:00	overnight
Serum	01-Nov-12	DRH072BS	15:05:00	18:00:00	18:25:00	6 hours
Urine	01-Nov-12	DRH072BU	14:30:00	17:50:00	18:25:00	6 hours
Serum	02-Nov-12	DRH087S	08:50:00	10:10:00	10:55:00	overnight
Urine	02-Nov-12	DRH087U	09:45:00	11:35:00	12:35:00	overnight
Serum	02-Nov-12	DRH088S	10:05:00	12:05:00	12:35:00	overnight
Urine	02-Nov-12	DRH088U	10:10:00	11:35:00	12:35:00	overnight
Serum	02-Nov-12	DRH089S	10:40:00	12:05:00	12:35:00	overnight
Urine	02-Nov-12	DRH089U	10:45:00	11:45:00	12:35:00	overnight
Serum	06-Nov-12	DRH090AS	09:20:00	10:50:00	11:15:00	overnight
Urine	06-Nov-12	DRH090AU	08:50:00	10:40:00	11:15:00	overnight
Serum	14-Nov-12	DRH091AS	09:00:00	10:15:00	10:35:00	overnight
Urine	14-Nov-12	DRH091AU	08:55:00	09:40:00	10:35:00	overnight
Serum	16-Nov-12	DRH092S	08:30:00	12:15:00	12:40:00	overnight
Urine	16-Nov-12	DRH092U	08:35:00	12:05:00	12:40:00	overnight
Serum	16-Nov-12	DRH093S	08:40:00	12:15:00	12:40:00	overnight
Urine	16-Nov-12	DRH093U	08:45:00	12:05:00	12:40:00	overnight
Serum	16-Nov-12	DRH094S	10:30:00	14:05:00	14:55:00	overnight
Urine	16-Nov-12	DRH094U	10:35:00	13:55:00	14:55:00	overnight
Serum	16-Nov-12	DRH095S	10:55:00	14:05:00	14:55:00	overnight
Urine	16-Nov-12	DRH095U	11:00:00	13:55:00	14:55:00	overnight

Serum	27-Nov-12	DRH075BS	10:05:00	12:15:00	12:45:00	overnight
Urine	27-Nov-12	DRH075BU	10:30:00	12:05:00	12:45:00	overnight
Serum	28-Nov-12	DRH091BS	09:15:00	11:40:00	12:10:00	overnight
Urine	28-Nov-12	DRH091BU	09:05:00	11:30:00	12:10:00	overnight
Serum	29-Nov-12	DRH047DS	09:10:00	10:30:00	11:20:00	overnight
Urine	29-Nov-12	DRH047DU	09:15:00	10:40:00	11:20:00	overnight
Serum	30-Nov-12	DRH096AS	12:15:00	15:20:00	15:40:00	6 hours
Urine	30-Nov-12	DRH096AU	14:50:00	15:10:00	15:40:00	8 hours
Serum	07-Dec-12	DRH049CS	12:55:00	15:25:00	15:50:00	5.5 hours
Urine	07-Dec-12	DRH049CU	13:00:00	15:15:00	15:50:00	5.5 hours
Serum	11-Dec-12	DRH097AS	07:35:00	10:35:00	11:40:00	overnight
Urine	11-Dec-12	DRH097AU	07:40:00	10:00:00	11:40:00	overnight
Serum	12-Dec-12	DRH098AS	07:00:00	09:25:00	10:25:00	overnight
Urine	12-Dec-12	DRH098AU	06:10:00	08:55:00	10:25:00	overnight
Serum	12-Dec-12	DRH100S	08:00:00	09:25:00	10:25:00	overnight
Urine	12-Dec-12	DRH100U	08:05:00	08:55:00	10:25:00	overnight
Serum	12-Dec-12	DRH101S	08:15:00	09:25:00	10:40:00	overnight
Urine	12-Dec-12	DRH101U	08:10:00	09:05:00	10:40:00	overnight
Serum	12-Dec-12	DRH042ES	16:15:00	17:25:00	17:45:00	6 hours
Urine	12-Dec-12	DRH042EU	16:00:00	17:15:00	17:45:00	6 hours
Serum	13-Dec-12	DRH102AS	05:55:00	09:00:00	09:50:00	overnight
Urine	13-Dec-12	DRH102AU	06:00:00	09:20:00	09:50:00	overnight
Serum	18-Dec-12	DRH103S	09:40:00	11:40:00	12:40:00	overnight
Urine	18-Dec-12	DRH103U	09:45:00	11:15:00	12:40:00	overnight
Serum	28-Dec-12	DRH029DS	09:00:00	10:05:00	10:30:00	overnight
Urine	28-Dec-12	DRH029DU	09:05:00	09:55:00	10:30:00	overnight
Serum	04-Jan-13	DRH098BS	06:00:00	08:30:00	08:55:00	overnight
Urine	04-Jan-13	DRH098BU	05:55:00	08:20:00	08:55:00	overnight
Serum	09-Jan-13	IHC141S	12:10:00	13:15:00	14:00:00	overnight
Urine	09-Jan-13	IHC141U	12:30:00	13:00:00	14:00:00	overnight
Serum	16-Jan-13	DRH104AS	10:00:00	12:20:00	13:05:00	overnight

Urine	16-Jan-13	DRH104AU	09:55:00	12:55:00	13:05:00	overnight
Serum	16-Jan-13	DRH105AS	11:00:00	12:20:00	13:05:00	overnight
Urine	16-Jan-13	DRH105AU	11:30:00	12:55:00	13:05:00	overnight
Serum	17-Jan-13	DRH106AS	09:05:00	11:40:00	13:05:00	overnight
Urine	17-Jan-13	DRH106AU	09:00:00	11:30:00	13:05:00	overnight
Serum	17-Jan-13	DRH039ES	10:05:00	11:40:00	13:05:00	overnight
Urine	17-Jan-13	DRH039EU	10:00:00	11:30:00	13:05:00	overnight
Serum	17-Jan-13	DRH085BS	14:40:00	16:10:00	16:45:00	6 hours
Urine	17-Jan-13	DRH085BU	14:35:00	15:55:00	16:45:00	6 hours
Serum	18-Jan-13	DRH071CS	10:35:00	12:50:00	13:30:00	overnight
Urine	18-Jan-13	DRH071CU	10:40:00	12:35:00	13:30:00	overnight
Serum	19-Jan-13	DRH090BS	10:10:00	11:15:00	11:30:00	overnight
Urine	19-Jan-13	DRH090BU	10:00:00	10:40:00	11:30:00	overnight
Serum	23-Jan-13	DRH200AS	06:25:00	09:45:00	10:45:00	overnight
Urine	23-Jan-13	DRH200AU	06:30:00	09:30:00	10:45:00	overnight
Serum	29-Jan-13	DRH104BS	09:10:00	11:00:00	11:45:00	overnight
Urine	29-Jan-13	DRH104BU	09:00:00	10:45:00	11:45:00	overnight
Serum	29-Jan-13	DRH049DS	07:40:00	11:00:00	11:45:00	overnight
Urine	29-Jan-13	DRH049DU	07:50:00	10:45:00	11:45:00	overnight
Serum	29-Jan-13	DRH043DS	12:25:00	14:30:00	15:20:00	6 hours
Urine	29-Jan-13	DRH043DU	12:30:00	14:40:00	15:20:00	6 hours
Serum	30-Jan-13	DRH105BS	10:35:00	12:45:00	13:20:00	overnight
Urine	30-Jan-13	DRH105BU	10:00:00	12:30:00	13:20:00	overnight
Serum	31-Jan-13	DRH106BS	10:10:00	12:45:00	13:20:00	overnight
Urine	31-Jan-13	DRH106BU	10:00:00	12:35:00	13:20:00	overnight
Serum	31-Jan-13	DRH039FS	11:05:00	12:45:00	13:20:00	overnight
Urine	31-Jan-13	DRH039FU	11:00:00	12:35:00	13:20:00	overnight
Serum	01-Feb-13	DRH108S	08:30:00	13:10:00	13:35:00	overnight
Urine	01-Feb-13	DRH108U	08:35:00	13:00:00	13:35:00	overnight
Serum	01-Feb-13	DRH107S	08:25:00	13:10:00	13:35:00	overnight
Urine	01-Feb-13	DRH107U	08:10:00	13:00:00	13:35:00	overnight

Serum	01-Feb-13	DRH097BS	09:45:00	13:40:00	14:20:00	overnight
Urine	01-Feb-13	DRH097BU	09:50:00	13:25:00	14:20:00	overnight
Serum	01-Feb-13	DRH096BS	12:05:00	14:05:00	15:35:00	eating mint sweets
Urine	01-Feb-13	DRH096BU	12:10:00	13:55:00	15:35:00	eating mint sweets
Serum	01-Feb-13	DRH102BS	11:55:00	14:05:00	15:35:00	overnight
Urine	01-Feb-13	DRH102BU	11:50:00	13:55:00	15:35:00	overnight
Serum	12-Feb-13	DRH086BS	11:00:00		12:10:00	overnight
Urine	12-Feb-13	DRH086BU	11:10:00		12:10:00	overnight
Serum	08-Feb-13	DRH018ES	09:00:00		10:30:00	overnight
Urine	08-Feb-13	DRH018EU	10:00:00		10:30:00	overnight
Serum	25-Feb-13	DRH200BS	13:50:00	15:10:00	16:10:00	6 hours
Urine	25-Feb-13	DRH200BU	13:50:00	14:45:00	16:10:00	6 hours
Serum	25-Feb-13	DRH098CS	14:00:00	15:10:00	16:10:00	6 hours
Urine	25-Feb-13	DRH098CU	14:05:00	14:45:00	16:10:00	6 hours
Serum	25-Feb-13	DRH042FS	13:40:00	15:10:00	16:10:00	6 hours
Urine	25-Feb-13	DRH042FU	13:30:00	14:45:00	16:10:00	6 hours

8.4 Complete Metabolite List			
Name	**Compound**	**Class**	**Description**
(+/-)-1-Phenylethylmercaptan	HMDB32467	Benzene and substituted derivates	Food additive
(+/-)-Dihydromintlactone	HMDB32219	Not classified	A food additive
(±)-(E)-3-Methyl-4-decen-1-yl acetate	HMDB32792	Fatty acyls	Found in herbs and spices
(±)-2-(3,4-Dihydroxyphenyl)-1,3-benzodioxole-5-carboxaldehyde	HMDB33088	Benzodioxoles	Found in herbs and spices
(±)-Glycerol 1-monophosphate K salt (1:2)	HMDB40362	Not classified	Food additive
(±)-Glycerol 1-monophosphate Mg salt (1:1)	HMDB40363	Not classified	Food additive
(±)-Sphaerosin	HMDB38128	Not classified	Found in the common bean
(1RS,2RS)-Guaiacylglycerol 1-glucoside	HMDB33300	Not classified	From Scotch pine needles
(1x,2x)-Guaiacylglycerol 2-glucoside	HMDB33301	Not classified	From Scotch pine needles
(1x,2x)-Guaiacylglycerol 3-glucoside	HMDB40600	Not classified	From Scotch pine needles
(2E,4Z,7Z)-2,4,7-Tridecatrienal	HMDB33545	Fatty acyls	Found in animal foods
(2E,4Z,7Z)-2,4,7-Tridecatrienal	HMDB33545	Fatty acyls	Found in animal foods
(2R,3R,4R)-2-Amino-4-hydroxy-3-methylpentanoic acid	HMDB29449	Fatty acyls	Found in herbs and spices
(3beta,5alpha,6beta,7alpha,22E,24R)-Ergosta-8,22-diene-3,5,6,7-tetrol	HMDB32107	Steroids and steroid derivatives	Found in mushrooms
(3E)-2-Propylpent-3-enoic acid	HMDB13904	Not classified	Metabolite of valproic acid, used in epilepsy treatemnt
(3S,5R,6R,7E)-3,5,6-Trihydroxy-7-megastigmen-9-one	HMDB38736	Not classified	Found in herbs and spices
(3xi,5Z)-1,5-Octadien-3-ol	HMDB30966	Fatty acyls	Found in crustaceans
(9xi,10xi,12xi)-9,10-Dihydroxy-12-octadecenoic acid	HMDB31679	Fatty Acyls	Found in fruits
(E)-5,8-Megastigmadien-4-one	HMDB34671	Carbonyl compounds	Found in fruits
(E)-5,8-Megastigmadien-4-one	HMDB34671	Carbonyl compounds	Found in fruit

(R)-(E)-4,7-Megastigmadien-9-one	HMDB35753	Prenol lipids	Flavouring agent
(R)-(E)-4,7-Megastigmadien-9-one	HMDB35753	Prenol lipids	Found in essential oils
(R)-3',7-Dihydroxy-2',4'-dimethoxyisoflavan	HMDB30717	Isoflavanoids	Found in the common bean
(R)C(R)S-S-Propylcysteine sulfoxide	HMDB29442	Carboxylic acids and derivatives	Found in onion family vegetables
(R,S)-Norlaudanosoline	HMDB12486	Not classified	A key intermediate in the synthesis of the benzylisoquinoline alkaloids
(S,E)-Filbertone	HMDB35242	Not classified	Found in nuts
[12]-Gingerol	HMDB36356	Benzene and substituted derivatives	Found in ginger
1-(2,4,5-Trimethoxyphenyl)-1,2-propanedione	HMDB31771	Benzene and substrituted derivatives	Found in herbs and spices
1-(2H-1,3-Benzodioxol-5-yl)-2-[2,6-dimethoxy-4-(prop-2-en-1-yl)phenoxy]propyl benzoate	HMDB39249	Not classified	Found in herbs and spices
1-(4-Methoxyphenyl)-2-nitroethylene	HMDB32595	Benzene and substituted derivatives	Used for the control of rice blast disease
1,1-Diethoxy-2,6-nonadiene	HMDB38078	Ethers	A flavouring ingredient
1,2,3,4-Tetrahydro-beta-carboline	HMDB12488	Indoles and derivatives	Found in chocolate and cocoa
1,2,3,4-Tetrahydro-beta-carboline	HMDB12488	Indoles and derivatives	A potential neuroactive alkaloid found in chocolate and cocoa
1,2-Benzisothiazol-3(2H)-one	HMDB34413	Benzothiazoles	An industrial biocide
1,2-Bis(1-ethoxyethoxy)propane	HMDB37163	Ethers	A flavouring agent
1,2-Epoxy-1,2,7,7',8,8',11',12'-octahydro-psi,psi-carotene	HMDB29854	Not classified	Found in tomatoes
1,4'-Bipiperidine-1'-carboxylic acid	HMDB60336	Piperidines	A piperidinecarboxylic acid
11-alpha-O-beta-D-Glucopyranosyl-16alpha-O-methylneoquassin	HMDB39773	Prenol lipids	Aconstituent of Quassia amara
12,13-DHOME	HMDB04705	Fatty Acyls	The epoxide hydrolase metabolite of the leukotoxin12,13-EpOME
13'-Carboxy-gamma-tocopherol	HMDB12557	Prenol lipids	A dehydrogenation carboxylate product of 13'-hydroxy-r-tocopherol
16,17-Dihydro-16a,17-dihydroxygibberellin A4 17-glucoside	HMDB40647	Prenol lipids	Found in cereals and cereal products

1-Acetoxy-2-hydroxy-5,12,15-heneicosatrien-4-one	HMDB35273	Fatty acyls	Found in fruits
1D-Myo-inositol 1,3,4,6-tetrakisphosphate	HMDB01187	Alcohols and polyols	A substrate for Tyrosine-protein kinase BTK and Inositol polyphosphate multikinase
1D-Myo-inositol 1,4,5,6-tetrakisphosphate	HMDB04527	Alcohols and polyols	Regulates chloride transport
1-Ethylhexyl tiglate	HMDB37626	Fatty acyls	A flavouring ingredient
1-Hydroxy-3,6,7-trimethoxy-2,8-diprenylxanthone	HMDB36597	Benzopyrans	Found in fruits
1-Penten-3-one	HMDB31607	Carbonyl compounds	Found in animal foods
2,3-Diaminopropionic acid	HMDB02006	Carboxylic acids and derivatives	A non-proteogenic amino acid found in antibiotics
2,3-Diethylpyrazine	HMDB41253	Diazines	Found in cereals and cereal products
2,3-Dihydrothiophene	HMDB33875	Dihydrothiophenes	A Maillard product
2,3-Octanedione	HMDB01568	Carbonyl compounds	Found in coffee and coffee products
2,4,4-Trimethylcyclopentanone	HMDB31197	Carbonyl compounds	Found in fats and oils
2,4-Diaminobutyric acid	HMDB02362	Carboxylic acids and derivatives	A non-physiological, cationic amino acid analogue, used in glioma treatment
2,4-Diisopropyl-3-methylphenol	HMDB29824	Benzene and substituted derivatives	Found in herbs and spices
2,4-Diisopropyl-3-methylphenol	HMDB29824	Benzene and substituted derivatives	Found in herbs and spices
2,4-Diisopropyl-5-methylphenol	HMDB29823	Prenol lipids	Found in herbs and spices
2,4-Diisopropyl-5-methylphenol	HMDB29823	Prenol lipids	Found in herbs and spices
2,5-Dichloro-4-oxohex-2-enedioate	HMDB60363	Keto acids and derivatives	Medium chain keto acid
2,5-Diethylpyrazine	HMDB36808	Diazines	Found in coffee and coffee products
2,5-Diisopropyl-3-methylphenol	HMDB29822	Prenol lipids	Found in herbs and spices
2,5-Diisopropyl-3-methylphenol	HMDB29822	Prenol lipids	Found in herbs and spices
2,6-Diisopropylnaphthalene	HMDB59902	Naphthalenes	Plant growth regulator
2,6-Dimethoxy-4-phenanthrenol	HMDB32890	Phenanthrenes and derivatives	Found in poppy seeds, and opium extracts
2,6-Dimethylbenzenethiol	HMDB32019	Benzene and substituted derivates	Food additive
2',7-Dihydroxy-4',6-dimethoxyisoflavan	HMDB33996	Isoflavanoids	Found in pulses
2-[(2-Furanylmethyl)thio]-6-methylpyrazine	HMDB36187	Thioethers	Present in pumpkin seed oil

23-trans-p-Coumaroyloxytormentic acid	HMDB40682	Prenol lipids	Found in fruits
28-Glucosylpomolate	HMDB38452	Prenol lipids	Found in herbs and spices
2-Arachidonylglycerol	HMDB11549	Glycerolipids	A monoacylglyceride and endogenous ligand for the cannabinoid receptors
2-Carboxy-4-dodecanolide	HMDB30987	Not classified	Found in milk and milk products
2-Carboxy-5,7-dimethyl-4-octanolide	HMDB30986	Lactones	Found in milk and milk products
2-Ethyl-3,(5 or 6)-dimethylpyrazine	HMDB32276	Diazines	Found in coffee and coffee products
2-Ethylbenzenethiol	HMDB41361	Benzene and substituted derivates	Flavouring ingredient in coffee
2-Ethylglutaric acid	HMDB59738	Not classified	A fatty acid containing a branched chain
2-Hexenyl acetate	HMDB40212	Carboxylic acids and derivatives	Found in fruits
2-Hydroxy-22-methyltetracosanoic acid	HMDB40909	Fatty acyls	Found in lanolin wool fat
2-Methyl-3 or 5 or 6-(furfurylthio)pyrazine (mixture of isomers)	HMDB32414	Thioethers	Food additive
2-Methylbutyroylcarnitine	HMDB00378	Fatty acyls	Not usually detected in normal individuals and its elevation suggests a deficiency of a dehydrogenase specific for isobutyryl-CoA
2-Methylbutyrylglycine	HMDB00339	Carboxylic acids and derivatives	An acyl glycine, normally a minor metabolite of fatty acids
2-Octen-4-one	HMDB31301	Carbonyl compounds	Found in cereal and cereal products
2-Octenal	HMDB30961	Carbonyl compounds	A flavouring ingredient
2-Octenoic acid	HMDB00392	Fatty acyls	Medium chain fatty acid
2-Propylsuccinic acid	HMDB61239	Not classified	Dicarboxylic acid
3,3',4,4'-Tetrachloroazobenzene	HMDB32856	Azobenzenes	An environmental pollutant arising from the soil degradation of DXZ32-P and related herbicides
3,4,5-Trimethoxycinnamic acid	HMDB02511	Cinnamic acids and derivatives	Found in pepper
3,4-Diethylthiophene	HMDB40236	Heteroaromatic compounds	Found in garden onion
3-Dehydrocarnitine	HMDB12154	Keto acids and derivatives	An intermediate in carnitine degradation
3'-Deoxyoleacein	HMDB37494	Benzene and substituted derivatives	Found in fats and oils
3'-Hydroxy-3,4,5,4'-tetramethoxystilbene	HMDB41653	Stilbenes	A polyphenol metabolite detected in biological fluids

3-Hydroxy-4-aminopyridine	HMDB60741	Pyridines and derivatives	A metabolite of dalfampridine, used in the management of multiple sclerosis
3-keto-2-Methylbutyrate	HMDB29172	Not classified	Used in the diagnosis of beta-ketothiolase deficiency
3-Methoxy-4,5-methylenedioxybenzoic acid	HMDB30800	Benzene and substituted derivatives	Found in green vegetables
3-Methoxymorphinan	HMDB14045	Morphinans	A metabolite of dextromethorphan
3-Methyladipic acid	HMDB00555	Fatty acyls	A metabolite of the catabolism of phytanic acid
3'-O-Methyl-(-)-epicatechin-7-O-sulphate	HMDB29177	Flavanoids	A urinary and gut-derived metabolite of epicatechin
3-Phenylpropyl hexanoate	HMDB36390	Fatty acyls	A flavouring ingredient
3-Succinoylpyridine	HMDB00992	Keto acids and derivatives	The byproduct of tobacco-specific N-nitrosamines generated by the enzyme cytochrome P 450 which catalyzes methylnitrosaminopyridylbutanone hydroxylation
3-Sulfodeoxycholic acid	HMDB02504	Steroids and steroid deivatives	Sulfated steroid, a sterol lipid containing a sulfate group
3-trans-Caffeoyltormentic acid	HMDB40650	Prenol lipids	Found in fruits
4-(2,6,6-Trimethylcyclohex-1-enyl)but-2-en-4-one	HMDB32541	Carbonyl compounds	Found in tea
4-(2,6,6-Trimethylcyclohex-1-enyl)but-2-en-4-one	HMDB32541	Carbonyl compounds	Found in tea
4-(3-Hydroxybutyl)-3,3,5-trimethylcyclohexanone	HMDB39805	Prenol lipids	Found in alcoholic beverages
4,10-Longipinanedione	HMDB32081	Prenol lipids	Found in herbs and spices
4',5-Dihydroxy-7-methoxy-6-methylflavone	HMDB29512	Flavonoids	Found in beverages
4-Hydroxy-benzenepropanedioate	HMDB59809	Benzene and substituted derivatives	An aromatic compound containing a benzene ring substituted by a hydroxyl group and an ester group
4-Hydroxystachydrine	HMDB29230	Carboxylic acids and derivatives	Biomarker of citrus consumption found in urine
4-Methylphenyl octanoate	HMDB37710	Benzene and substituted derivatives	Flavouring ingredient
4-Phosphopantothenoylcysteine	HMDB01117	Carboxylic acids and derivatives	An intermediate in the biosynthetic pathway that converts pantothenate (vitamin B5) into coenzyme A (CoA).

4-Thiocyanatophenol	HMDB40578	Benzene and substituted derivatives	From fruits and vegetables
4-Thiocyanatophenol	HMDB40578	Benzene and substituted derivatives	Found in fruits
5,6,8-Trihydroxy-2-methylbenzo[g]chromen-4-one	HMDB33911	Naphthopyrans	Found in coffee and coffee products
5a-Cholest-8-en-3b-ol	HMDB06841	Steroids and steroid derivatives	An intermediate of cholesterol synthesis
5alpha-Cholestanone	HMDB00871	Steroids and steroid derivatives	An oxidation product of coprosterol
5-Aminoimidazole	HMDB03929	Azoles	An intermediate in the formation of purines
5-beta-Cholestan-3-one	HMDB59604	Steroids and steroid derivatives	Part of the Primary bile acid biosynthesis, and steroid hormone biosynthesis pathways
5beta-Cholestanone	HMDB11182	Steroids and steroid derivatives	An oxidation product of coprosterol
5-Decanoyl-2-nonylpyridine	HMDB35516	Carbonyl compound	Found in herbs and spices
5-Methyl-5-hepten-2-one	HMDB31591	Carbonyl compounds	Found in root vegetables
5-Octen-2-one	HMDB35390	Carbonyl compounds	Found in citrus
6-Hydroxy-2,6-dimethyl-2,7-octadien-4-one	HMDB34670	Prenol lipids	Found in citrus
6-Mercaptopurine riboside	HMDB61269	Imidazopyrimidines	A metabolite of mercaptopurine
6-Octenal	HMDB39769	Carbonyl compounds	Food additive
6-Thioinosinic acid	HMDB60791	Carbohydrates and carbohydrate conjugates	A metabolite of azathioprine
6-trans-Leukotriene B4	HMDB05087	Fatty Acyls	An enzymatic metabolite of leukotriene B4(LTB4).
7(14)-Bisabolene-2,3,10,11-tetrol	HMDB35918	Not classified	A mycotoxin of Fusarium sambucinum
7,9-Illudadiene-3,14-diol	HMDB38795	Not classified	Found in mushrooms
7-Aminoflunitrazepam	HMDB41818	Benzodiazepines	A pharmacologically-active metabolite of flunitrazepam
7-Hydroxycostal	HMDB35202	Not classified	Found in potato
8,15-Isopimaradien-18-oic acid	HMDB35692	Prenol lipids	Found in pine tree resin
8-Hydroxyguanosine	HMDB02044	Purine nucleosides	A marker for measuring the rate of oxidative damage to nucleic acids and lipids
8-Hydroxyhesperetin 7-[6-acetylglucosyl-(1->2)-glucoside]	HMDB41232	Flavonoids	Found in tea
9,10-DHOME	HMDB04704	Fatty Acyls	A derivative of linoleic acid diol

Acetamide	HMDB31645	Carboxylic acids and derivatives	Found in red beetroot
Acetyl-N-formyl-5-methoxykynurenamine	HMDB04259	Benzene and substituted derivatives	Results from the oxidative cleavage of the pyrrole ring during melatonin oxidation by myeloperoxidase
Adouetine Y	HMDB34101	Not classified	Found in tea
Adrenochrome	HMDB12884	Not classified	A pigment obtained by the oxidation of adrenaline
Alfafuran	HMDB38677	2-arylbenzofuran flavonoids	Found in pulses
Alfalone	HMDB38811	Isoflavonoids	Found in alfalfa
Alpha-Carboxy-delta-decalactone	HMDB30985	Lactones	Found in milk and milk products
alpha-Damascone	HMDB36027	Not classified	Found in tea
alpha-Damascone	HMDB36027	Not classified	Found in tea
alpha-Methylstyrene	HMDB59899	Benzene and substituted derivatives	Used in the manufacture of plasticisers, resins and polymers
Alpha-N-Phenylacetyl-L-glutamine	HMDB06344	Carboxylic acids and derivatives	A product formed by the conjugation of phenylacetate and glutamine
Alternariol	HMDB30831	Coumarins and derivatives	A mycotoxin produced by Alternaria fungi
Alternariol	HMDB30831	Coumarins and derivatives	Found in mushrooms
Ambenonium	HMDB15254	Carboxylic acids and derivatives	A cholinesterase inhibitor used in the management of myasthenia gravis
Aminomalonic acid	HMDB01147	Carboxylic acids and derivatives	Isolated from proteins of E.coli and atherosclerotic plaque
Ammonium peroxydisulfate	HMDB37638	Non-metal oxoanionic compounds	A strong oxidising agent
Anguidol	HMDB35846	Prenol lipids	Produced by Fusarium roseum, Fusarium equiseti and Fusarium sporotrichiella
Apigenin 7-sulfate	HMDB37851	Flavonoids	Isolated from Bixa orellana
Arginyl-Phenylalanine	HMDB28716	Carboxylic acids and derivatives	An incomplete breakdown product of protein digestion or protein catabolism
Artabsinolide D	HMDB34941	Prenol lipids	Found in alcoholic beverages
Artocarpetin B	HMDB33148	Flavanoids	Found in fruits
Artonol D	HMDB30495	Not classified	Found in breadfruit

Ascochitine	HMDB30145	Benzene and substituted derivates	Causes rot in broad bean
Austalide C	HMDB34075	Benzopyrans	A metabolite of Aspergillus ustus
Benzyl methyl sulfide	HMDB31314	Benzene and substituted derivates	Found in animal foods
Beta-Citryl-L-glutamic acid	HMDB13220	Carboxylic acids and derivatives	A derivative of glutamic acid
Betahistine	HMDB15644	Amines	An anti-vertigo drug
Biliverdin	HMDB01008	Tetrapyrroles and derivatives	A byproduct of hemoglobin breakdown
Bisbynin	HMDB41324	Not classified	Found in cereals and cereal products
Bn-NCC-2	HMDB38230	Tetrapyrroles and derivatives	Found in brassicas
Butyl (S)-3-hydroxybutyrate [arabinosyl-(1->6)-glucoside]	HMDB39214	Not classified	Found in fruits
Butyl 1-(methylthio)propyl disulfide	HMDB33049	Organic disulfides	Found in onion family vegetables
Butyl 1-(methylthio)propyl disulfide	HMDB33049	Organic disulphides	From fruits and vegetables
Calystegine A3	HMDB38593	Tropane alkaloids	Found in alcoholic beverages
Calystegine A6	HMDB31345	Tropane alkaloids	Found in coffee
Calystegine A7	HMDB36384	Not classified	An alkaloid from the roots of Lycium chinense
Canescein	HMDB29313	Steroids and steroid derivatives	Isolated from Convallaria majalis
Canesceol	HMDB34084	Steroids and steroid derivatives	Isolated from Convallaria majalis
Casomorphin	HMDB59787	Not classified	A protein found in the milk of mammals
Ceanothine C	HMDB29340	Carboxylic acids and derivatives	Found in tea
Ceanothine D	HMDB29341	Carboxylic acids and derivatives	Found in tea
Ceanothine E	HMDB29342	Not classified	Found in tea
Cefuroxime	HMDB15244	Lactams	A broad-spectrum cephalosporin antibiotic resistant to beta-lactamase
Cellobiose	HMDB00055	Carbohydrates and carbohydrate conjugates	Obtained from the partial hydrolysis of cellulose
Cerebroside B	HMDB35990	Sphingolipids	Found in mushrooms
Chlophedianol	HMDB15585	Benzene and substituted derivatives	Centrally acting cough suppressant
Chlordiazepoxide	HMDB14618	Benzodiazepines	An anxiolytic benzodiazepine derivative
Chloromethyl methyl ether	HMDB31332	Ethers	used to modify ion-exchange membranes used in

			the production of grapefruit juice
Chlorprothixene	HMDB15369	Benzothiopyrans	An antipsychotic medication
Chlorprothixene	HMDB15369	Benzothiopyrans	An antipsychotic drug of the thioxanthene (tricyclic) class
Cholesterol	HMDB00067	Steroids and steroid derivatives	A sterol (a combination steroid and alcohol) and a lipid found in the cell membranes of all body tissues, and transported in the blood plasma of all animals
Cinncassiol D2 glucoside	HMDB34679	Prenol lipids	Found in herbs and spices
cis-3-Hexenyl acetate	HMDB40215	Carboxylic acids and derivatives	Found in dill, green tea and fruit volatiles
Citalopram N-oxide	HMDB60654	Not classified	Metabolite of citalopram
Citalopram propionic acid	HMDB60463	Not classified	A metabolite of citalopram
Citric acid	HMDB00094	Carboxylic acids and derivatives	A weak acid that is formed in the tricarboxylic acid cycle or that may be introduced with diet
Citronellyl propionate	HMDB37226	Fatty acyls	Found in garden tomato
Coniferan	HMDB31741	Not classified	A food additive
CPA(18:2(9Z,12Z)/0:0)	HMDB07007	Lineolic acid and derivatives	A cyclic phosphatidic acid or cyclic lysophosphatidic acid
Cyclamic acid	HMDB31340	Sulfamic acid derivatives	An artificial sweetening agent
Cycloartanyl ferulate	HMDB36295	Not classified	Found in cereal and cereal products
Cyclohexaneacetic acid	HMDB31403	Carboxylic acids and derivatives	Flavouring ingredient
Cynaratriol	HMDB34983	Prenol lipids	Found in cardoon
Cyromazine	HMDB29862	Triazines	An insect growth regulator
Cysteinyl-Methionine	HMDB28781	Carboxylic acids and derivatives	A dipeptide composed of cysteine and methionine
Dambonitol	HMDB33942	Alcohols and polyols	Latex used for manufacture of chewing gum
Delavirdine	HMDB14843	Diazinanes	A non-nucleoside reverse transcriptase inhibitor with activity specific for HIV-1
Desmethyl frovatriptan	HMDB60815	Indoles and derivatives	A metabolite of frovatriptan, used in the treatment of migraine
Desvenlafaxine	HMDB15646	Not classified	Metabolite of venlafaxine

Dextrorphan	HMDB60552	Morphinans	A metabolite of dextromethorphan
Dezocine	HMDB15340	Tetralins	A partial opiate drug
Dhurrin	HMDB60471	Carbohydrates and carbohydrate conjugates	Found in plants
Di-2-propenyl tetrasulfide	HMDB33202	Sulfenyl compounds	Found in onion family vegetables
Dibutyl decanedioate	HMDB41220	Fatty Acyls	Used in fruit flavouring
Difluorodeoxyuridine monophosphate	HMDB61204	Pyrimidine nucleosides	Metabolite of gemcitabine
Dihydro-5-Hydroxyrofecoxib	HMDB61182	Dihydrothiophines	Found in animal foods
Dihydromethysticin	HMDB30791	Not classified	Found in beverages
Dihydrophaseic acid	HMDB38660	Prenol lipids	Found in coconut and French beans
Dihydroxybutanoic acid	HMDB00360	Fatty acyls	An organic acid that is a major component of CSF
di-Hydroxymelatonin	HMDB61136	Indoles and derivatives	Metabolite of melatonin
Dimethadione	HMDB61093	Azolines	aAmetabolite of trimethadione, an oxazolidinedione anticonvulsant
Dimethyl sulfoxide	HMDB02151	Sulfoxides	A key dipolar aprotic solvent
Dimethylprotoporphyrin IX dimethyl ester	HMDB00810	Tetrapyrroles and derivatives	The hepatic pigment accumulated as a consequence of the self-catalyzed destruction of cytochrome P-450 by norethisterone
Diphenhydramine N-glucuronide	HMDB60897	Carbohydrates and carbohydrate conjugates	A metabolite of diphenhydramine
Divinyl sulfide	HMDB33922	Thioethers	Found in onion family vegetables
D-Myo-inositol 3,4,5,6-tetrakisphosphate	HMDB03848	Alcohols and polyols	Has a direct biphasic (activation/inhibition) effect on an epithelial calcium-activated chloride channel
Doristerol	HMDB29815	Steroids and steroid derivatives	Found in root vegetables
Doxepin	HMDB15273	Benzoxepines	Tricyclic antidepressant
Dutasteride	HMDB15258	Steroids and steroid derivatives	5-alpha-reductase inhibitor
Edulan I	HMDB34959	Benzopyrans	Flavour constituent in purple passion fruit
Edulan I	HMDB34959	Benzopyrans	Found in fruit
Eicosane	HMDB59909	Alkanes	Acyclic hydrocarbon
ent-8(17),13(16),14-Labdatrien-18-oic acid	HMDB39486	Prenol lipids	Found in fruits
Epidihydrophaseic acid	HMDB38661	Prenol lipids	Found in pulses

Erythritol	HMDB02994	Carbohydrates and carbohydrate conjugates	Found in wine, sake, beer, water melon, pear, grape and soy sauce
Etamiphylline	HMDB41889	Not available (Super Class Alkaloids and derivatives)	Agent for the treatment of asthma
Ethyl (±)-2-methyl-4-pentenoate	HMDB29761	Fatty acyls	Flavouring agent
Ethyl 1-(propylthio)propyl disulfide	HMDB33048	Organic disulphides	From fruits and vegetables
Ethyl 1-(propylthio)propyl disulfide	HMDB33048	Organic disulfides	Found in fruits
Ethyl 10-undecenoate	HMDB34286	Fatty acyls	Found in alcoholic beverages
Ethyl 2E-hexenoate	HMDB32268	Fatty acyls	Flavouring agent
Ethyl beta-D-glucopyranoside	HMDB29968	Carbohydrates and carbohydrate conjugates	Found in pulses
Eucaglobulin	HMDB36742	Saccharolipids	A constituent of Eucalyptus globulus
Fagomine	HMDB33453	Piperidines	Found in fruits
Ferrocytochrome	HMDB12947	Not classified	A cytochrome containing reduced iron
Fluorescein	HMDB14831	Benzopyrans	A phthalic indicator dye that appears yellow-green in normal tear film and bright green in a more alkaline medium
Fluvoxamine	HMDB14322	Benzene and substituted derivatives	An antidepressant which functions pharmacologically as a selective serotonin reuptake inhibitor
Fructose	HMDB00660	Carbohydrates and carbohydrate conjugates	A simple monosaccharide found in fruits and berries
Galactose	HMDB00143	Carbohydrates and carbohydrate conjugates	An energy-providing nutrient
Gentisin	HMDB31760	Benzopyrans	Found in alcoholic beverages
Germacrone 4,5-epoxide	HMDB35889	Prenol lipids	Found in tumeric
Glucuronic acid	HMDB00127	Carbohydrates and carbohydrate conjugates	Synthesised in the uronic acid pathway
Glycerol	HMDB00131	Carbohydrates and carbohydrate conjugates	An important component of triglycerides and phospholipids
Glycyrin	HMDB33712	Isoflavanoids	Found in root vegetables
Goshonoside F1	HMDB38539	Not classified	Found in fruits
Goshonoside F2	HMDB38540	Not classified	Found in fruits

Guanethidine	HMDB15301	Guanidines	An antihypertensive agent
Gyromitrin	HMDB33952	Hydrazines and derivatives	Found in mushrooms
Hippuric acid	HMDB00714	Benzene and substituted derivatives	An acyl glycine formed by the conjugation of benzoic acid with glycine
Hordatine B	HMDB30459	2-arylbenzofuran flavonoids	Found in barley
Hydroxy-alpha-sanshool	HMDB29567	Alcohols and polyols	Found in herbs and spices
Hydroxylamine	HMDB03338	Homogenous other non-metal compounds	Used in organic synthesis and as a reducing agent
Imidazole-4-acetaldehyde	HMDB03905	Azoles	Aldehyde metabolite of histamine
Imidazole-4-acetaldehyde	HMDB03905	Azoles	A metabolite of histamine
Imidazoleacetic acid riboside	HMDB02331	Imidazole ribonucleosides and ribonucleotides	A metabolite of imidazoleacetic acid, histamine's oxidative metabolite
Indane	HMDB59837	Indanes	A hydrocarbon petrochemical compound
Inositol 1,3,4,5-tetraphosphate	HMDB01059	Alcohols and polyols	A common regulator in calcium homeostasis
Isogentisin	HMDB30871	Benzopyrans	Found in alcoholic beverages
Isomucronulatol	HMDB33189	Not classified	Found in the common bean
Isospirene	HMDB36022	Dihydrofurans	A fragrance ingredient
Isospirene	HMDB36022	Dihydrofurans	Fragrance ingredient
Isotridecanol	Not classified	Not classified	Fatty alcohol
Kanokoside A	HMDB35635	Not classified	Found in fats and oils
Kanzonol G	HMDB40613	Isoflavanoids	Found in herbs and spices
Kanzonol O	HMDB41102	Not classified	Found in herbs and spices
L-2,4-diaminobutyric acid	HMDB06284	Carboxylic acids and derivatives	a component of branched-chain amino acid biosynthesis and metabolism
Lactose	HMDB00186	Carbohydrates and carbohydrate conjugates	The major sugar present in milk
Lathosterol	HMDB01170	Steroids and steroid derivatives	A sterol (a combination steroid and alcohol) and a lipid found in the cell membranes of all body tissues, and transported in the blood plasma of all animals
Levorphanol	HMDB14992	Morphinans	A narcotic analgesic

Licoricone	HMDB29515	Isoflavanoids	Found in herbs and spices
Lisdexamfetamine	HMDB15385	Carboxylic acids and derivatives	Agent for the treatment of attention deficit hyperactivity disorder
Lithocholate 3-O-glucuronide	HMDB02513	Steroids and steroid derivatives	Found in bile
Loratadine	HMDB05000	Benzocyclohepatapyridines	Tricyclic antihistamine
Lucyoside Q	HMDB29621	Prenol lipids	Found in fruits
Luteolin 4'-sulfate	HMDB38471	Flavonoids	Found in carrot
LysoPE(0:0/24:6(6Z,9Z,12Z,15Z,18Z,21Z))	HMDB11499	Glycerophospholipids	A lysophospholipid
LysoPE(24:6(6Z,9Z,12Z,15Z,18Z,21Z)/0:0)	HMDB11529	Glycerophospholipids	A lysophospholipid
Lysyl-Proline	HMDB28959	Carboxylic acids and derivatives	An incomplete breakdown product of protein digestion or protein catabolism
Lysyl-Valine	HMDB28964	Carboxylic acids and derivatives	A dipeptide composed of lysine and valine
Malonic acid	HMDB00691	Carboxylic acids and derivatives	Acts against succinate dehydrogenase (complex II) in the respiratory electron transport chain
Marasmene	HMDB36035	Furofurans	Found in mushrooms
Maslinic acid 3-O-b-D-glucoside	HMDB29606	Not classified	Found in fruits
Matricarin	HMDB35790	Prenol lipids	Found in fats and oils
Medicanine	HMDB38625	Carboxylic acids and derivatives	Found in pulses
Mesoporphyrin IX	HMDB02379	Tetrapyrroles and derivatives	Porphyrin that inhibits interferon-gamma and interleukin-6 production
Methionyl-Cysteine	HMDB28970	Carboxylic acids and derivatives	A dipeptide composed of methionine and cysteine
Methoxypyrazine	HMDB33156	Diazines	A flavouring agent
Methyl (E)-2-dodecenoate	HMDB31028	Fatty acyls	Found in pomes
Methyl acrylate-divinylbenzene, completely hydrolyzed, copolymer	HMDB32389	Pyridines and derivatives	Used as a food additive
Methyl n-formylanthranilate	HMDB32398	Benzene and substituted derivatives	A food additive
Methyl propenyl ketone	HMDB01184	Carbonyl compounds	A volatile organic compound
Methyl salicylate O-[rhamnosyl-(1->6)-glucoside]	HMDB33138	Carbohydrates and carbohydrate conjugates	Found in fruits
Methyltestosterone	HMDB15655	Steroids and steroid derivatives	Anabolic sterois

MG(0:0/20:4(8Z,11Z,14Z,17Z)/0:0)	HMDB11549	Glycerolipids	A monoacylglyceride
MG(20:4(5Z,8Z,11Z,14Z)/0:0/0:0)	HMDB11578	Glycerolipids	A monoacylglyceride
MG(20:4(8Z,11Z,14Z,17Z)/0:0/0:0)	HMDB11579	Glycerolipids	A monoacylglyceride
Mianserin	HMDB15620	Benzazepines	A tetracyclic compound with antidepressant effects
Momordicin II	HMDB35966	Steroids and steroid derivatives	Found in bitter gourd
Momordicoside L	HMDB35917	Prenol lipids	Found in bitter gourd
Mono-methyl-adipate	HMDB59722	Fatty acyls	Medium-chain fatty acids
Moreollin	HMDB30795	Not classified	Found in fruits
Musabalbisiane B	HMDB38681	Prenol lipids	Found in fruits
Myo-inositol	HMDB00211	Alcohols and polyols	A product of glycerophospholipid metabolism
Myricetin 3,3'-digalactoside	HMDB37850	Not classified	Found in herbs and spices
N-(1-Deoxy-1-fructosyl)methionine	HMDB37841	Carbohydrates and carbohydrate conjugates	Food additive
N-(2-Hydroxyethyl)-morpholine N-oxide	HMDB61157	Oxazinanes	A metabolite of mycophenylate mofetil
N4-Acetylsulfamethoxazole	HMDB13854	Benzene and substituted derivatives	A metabolite of Sulfamethoxazole
N-Acetylglutamine	HMDB06029	Carboxylic acids and derivatives	From fruits and vegetables
N-Acetylvaline	HMDB11757	Carboxylic acids and derivatives	A derivative of valine
N-Acryloylglycine	HMDB01843	Carboxylic acids and derivatives	An acyl glycine, normally a minor metabolite of fatty acids
N-Desmethylvenlafaxine	HMDB13892	Not classified	Metabolite of venlafaxine
N-Nitroso-pyrrolidine	HMDB31642	Pyrrolidines	Found in animal foods
Noroxymorphone	HMDB61073	Morphinans	A metabolite of oxymorphone
NTP	HMDB60500	c-glycosyl compounds	Provides energy and a phosphate group for phosphorylations
Octadecanedioic acid	HMDB00782	Fatty Acyls	A long-chain dicarboxylic acid
Octanoic acid	HMDB59831	Fatty acyls	A carboxylic ester derivative of a fatty acid
O-Desmethylvenlafaxine	HMDB60532	Not classified	Metabolite of venlafaxine
Oleic acid	HMDB00207	Fatty acyls	An unsaturated fatty acid that is the most widely distributed and abundant fatty acid in nature

Oltipraz	HMDB41967	Diazines	A dithiole derivative and a schistosomicide
Ortho-hydroxyatorvastatin	HMDB61015	Not classified	A metabolite of atorvastatin
Oxacyclotetradecan-2-one	HMDB40451	Macrolides and analogues	Found in fats and oils
Oxaprozin	HMDB15126	Azoles	An anti-inflammatory medication
Oxytetracycline	HMDB14733	Tetracyclines	Isolated from the actinomycete streptomyces rimosus, used as an antibiotic
Para-hydroxyatorvastatin	HMDB61014	Not classified	A metabolite of atorvastatin
PC(14:0/18:0)	HMDB07871	Glycerophospholipids	A glycerophospholipid in which a phosphorylcholine moiety occupies a glycerol substitution site
PC(16:0/16:0)	HMDB00564	Glycerophospholipids	A glycerophospholipid in which a phosphorylcholine moiety occupies a glycerol substitution site
PC(18:0/14:0)	HMDB08031	Glycerophospholipids	A glycerophospholipid in which a phosphorylcholine moiety occupies a glycerol substitution site
p-Chlorobenzene sulfonyl urea	HMDB14026	Benzene and substituted derivatives	Found in individuals that have used or taken chlorpropamide, used in diabetes mellitus treatment
PE(15:0/20:0)	HMDB08899	Glycerophospholipids	A glycerophospholipid in which a phosphorylethanolamine moiety occupies a glycerol substitution site
PE(20:0/15:0)	HMDB09219	Glycerophospholipids	A glycerophospholipid in which a phosphorylethanolamine moiety occupies a glycerol substitution site
PE(20:4(8Z,11Z,14Z,17Z)/P-18:1(9Z))	HMDB09447	Glycerophospholipids	A glycerophospholipid in which a phosphorylethanolamine moiety occupies a glycerol substitution site
PE(22:5(4Z,7Z,10Z,13Z,16Z)/P-16:0)	HMDB09642	Glycerophospholipids	A glycerophospholipid in which a phosphorylethanolamine moiety occupies a glycerol substitution site
PE(P-16:0/22:5(4Z,7Z,10Z,13Z,16Z))	HMDB11359	Glycerophospholipids	A glycerophospholipid in which a phosphorylethanolamine moiety occupies a

			glycerol substitution site
PE(P-18:1(9Z)/20:4(5Z,8Z,11Z,14Z))	HMDB11451	Glycerophospholipids	A glycerophospholipid in which a phosphorylethanolamine moiety occupies a glycerol substitution site
Pentanoic acid	HMDB00892	Fatty Acyls	Straight chain fatty acid
Peroxynitrite	HMDB02179	Non-metal oxoanionic compounds	A potent oxidant synthesised by the cell during its normal metabolism
Persenone A	HMDB36568	Not classified	Found in fruits
Phenelzine	HMDB14918	Benzene and substituted derivatives	An irreversible non-selective inhibitor of monoamine oxidase, used in depression
Phenylalanyl-Arginine	HMDB28989	Not classified	An incomplete breakdown product of protein digestion or protein catabolism
Phosphate	HMDB01429	Non-metal oxoanionic compounds	A salt of phosphoric acid
p-HPEA-EDA	HMDB29305	Benzene and substituted derivatives	Found in olive
Phytoene 1,2-epoxide	HMDB36875	Prenol lipids	Found in tomatoes
Pimelic acid	HMDB00857	Fatty acyls	A group of compounds that are derivatives of heptanedioic acid with the general formula R-C7H11O4
Pipermethystine	HMDB33486	Benzene and substituted derivatives	An alkaloid from the leaves of kava
Pivaloylcarnitine	HMDB41993	Fatty acyls	An acyl carnitine
Porric acid C	HMDB31898	Benzofurans	Found in onion-family vegetables
Prolyl-Lysine	HMDB29022	Not classified	An incomplete breakdown product of protein digestion or protein catabolism
Propanetricarboxylic acid	HMDB31193	Carboxylic acids and derivatives	Found in corn
Protoporphyrinogen IX	HMDB01097	Tetrapyrroles and derivatives	An intermediate in heme synthesis
Ptelatoside A	HMDB32600	Not classified	Found in green vegetables
Pyridoxamine	HMDB01431	Pyridines and derivatives	The 4-aminomethyl form of vitamin B6
Pyroglutamic acid	HMDB00267	Carboxylic acids and derivatives	A cyclised derivative of L-glutamic acid
Pyrrolidonecarboxylic acid	HMDB00805	Carboxylic acids and derivatives	A cyclic derivative of glutamic acid
Quercetin 3-(3",6"-di-p-coumarylglucoside)	HMDB37373	Not classified	Found in Scotch pine

Retinyl ester	HMDB03598	Prenol lipids	The storage form of vitamin A
Rhazidigenine Nb-oxide	HMDB30263	Indoles and derivatives	An alkaloid from the bark of Aspidosperma quebracho-blanco
Rhodinyl propionate	HMDB37187	Fatty acyls	A flavouring ingredient
Rishitinol	HMDB38190	Not classified	Found in alcoholic beverages
Roxatidine acetate	HMDB15695	Piperidines	A drug metabolite
S-Acetyldihydrolipoamide	HMDB01526	Fatty acyls	An intermediate in alanine, aspartate and pyruvate metabolism and glycolysis/gluconeogenesis
S-Acetyldihydrolipoamide-E	HMDB06878	Fatty acyls	The acetyl thioester of the reduced lipoyllysine residue in dihydrolipoyllysine-residue acetyltransferase
S-aminomethyldihydrolipoamide	HMDB06239	Fatty acyls	An intermediate in the glycine, serine, threonine metabolism pathway
Sayanedin	HMDB30718	Isoflavonoids	Found in the common pea
Scorzonoside	HMDB38718	2-arylbenzofuran flavonoids	Found in coffee and coffee products
Serine	HMDB00187	Carboxylic acids and derivatives	A non-essential amino acid derived from glycine
S-Furanopetasitin	HMDB36131	Prenol lipids	Found in herbaceous plants
Sibutramine	HMDB15237	Benzene and substituted derivatives	Agent for the treatment of obesity
Soyasapogenol B 3-O-b-D-glucuronide	HMDB39329	Not classified	Formed by acid or alkaline hydrolysis of soyasaponins
Spirapril	HMDB15438	Carboxylic acids and derivatives	An ACE inhibitor antihypertensive drug used to treat hypertension
Sudachiin B	HMDB39087	Flavanoids	Found in citrus
Sudachiin C	HMDB39088	Not classified	Found in citrus
Sugeonol	HMDB41036	Prenol lipids	Found in root vegetables
Sulcatone	HMDB35915	Carbonyl compounds	Found in citrus
Sumatriptan	HMDB05037	Indoles and derivatives	Used in the treatment of migraine to stabilise serotonin levels in the brain
Taxiphyllin	HMDB30704	Carbohydrates and carbohydrate conjugates	Found in plants
Teniposide catechol derivative	HMDB61336	Lignan lactones	A semisynthetic derivative of podophyllotoxin

Tenofovir Monophosphate	HMDB61280	Imidazopyimidines	Belongs to a class of antiretroviral drugs known as nucleotide analogue reverse transcriptase inhibitors
Tetrahydro-2,5-furan-diacetic acid	HMDB59767	Dicarboxylic acids and derivatives	A dicarboxylic acid
TG(14:0/14:1(9Z)/18:4(6Z,9Z,12Z,15Z))	HMDB42295	Not classified	A dimyristoleic acid triglyceride
TG(14:0/18:4(6Z,9Z,12Z,15Z)/14:1(9Z))	HMDB42788	Not classified	A dimyristoleic acid triglyceride
TG(14:1(9Z)/14:0/18:4(6Z,9Z,12Z,15Z))	HMDB47741	Not classified	A dimyristoleic acid triglyceride
TG(14:1(9Z)/14:1(9Z)/18:3(6Z,9Z,12Z))	HMDB47894	Not classified	A dimyristoleic acid triglyceride
TG(14:1(9Z)/14:1(9Z)/18:3(9Z,12Z,15Z))	HMDB47901	Not classified	A dimyristoleic acid triglyceride
TG(14:1(9Z)/18:3(6Z,9Z,12Z)/14:1(9Z))	HMDB48092	Not classified	A dimyristoleic acid triglyceride
TG(14:1(9Z)/18:3(9Z,12Z,15Z)/14:1(9Z))	HMDB48253	Not classified	A dimyristoleic acid triglyceride
Thiabendazole	HMDB14868	Benzimidazoles	Treatment against nematodes
Threonine	HMDB00167	Fatty acyls	Essential amino acid
Tiludronate	HMDB15265	Organic phosphonic acids and derivatives	A bisphosphonate medication
Trabectedin metabolite M8b (ET-729)	HMDB61254	Benzene and substituted derivatives	A marine derived antitumoral agent
Tracheloside	HMDB30557	Not classified	Found in fats and oils
Tramadol	HMDB14339	Benzene and substituted derivatives	Narcotic analgesic
trans-2-Octenoic acid	HMDB01568	Fatty acyl	Medium chain fatty acid
Turicine	HMDB29409	Carboxylic acids and derivatives	A constituent of jack bean
Urea	HMDB00294	Organic carbonic acids and derivatives	Formed in the liver from ammonia
Uric acid	HMDB00289	Not available (Super Class Alkanoids and derivative)	A heterocyclic purine derivative that is the final oxidation product of purine metabolism
Valerylcarnitine	HMDB13128	Fatty acyls	An acyl carnitine
Valerylglycine	HMDB00927	Carboxylic acids and derivatives	An acyl glycine, normally a minor metabolite of fatty acids
Valproic acid glucuronide	HMDB00901	Carbohydrates and carbohydrate conjugates	The glucuronidation product of valproic acid
Valpronic acid beta-O-glucuronide	HMDB60889	O-glucuronides	The glucuronidation product of valproic acid
Valyl-Lysine	HMDB29132	Not classified	A dipeptide composed of valine and lysine
Vignatic acid A	HMDB33599	Carboxylic acids and derivatives	Found in pulses

xi-Tetrahydro-3-propyl-2H-pyran-2-one	HMDB37630	Lactones	Found in fruits
Yucalexin A16	HMDB36775	Prenol lipids	Found in root vegetables
Yucalexin B14	HMDB36709	Prenol lipids	Found in root vegetables
Zerumbone oxide	HMDB36466	Carbonyl compounds	Found in herbs and spices
Zileuton sulfoxide	HMDB13969	Not classified	Drug metabolite used in the treatment of asthma